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PYRUVATE OXIDATION AND THE CITRIC ACID CYCLE[1]

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From the Department of Biochemistry, The University of Chicago

Submitted for publication June 9, 1941

Knowledge of the mechanisms by which pyruvic acid is oxidized in living cells is fundamental to an understanding of a great variety of metabolic reactions. This simple keto acid is apparently not only an intermediate in the metabolism of carbohydrate, but of protein and also possibly fat. It is converted into acetoacetic acid in the liver, participates in the transaminating reactions occurring in many tissues, and is intimately concerned, as we have known for a long time, with the cellular functions of vitamin B_1.

A critical survey of the numerous reactions which are known to occur when pyruvic acid is metabolized by various types of living cells would embrace the whole field of metabolic investigation. The present discussion is concerned only with a few of the more recent investigations of the metabolism of pyruvic acid. Since the mechanisms involved in the general reaction,

$$\text{pyruvic acid} + 2\tfrac{1}{2}O_2 \longrightarrow 3CO_2 + 2\ H_2O,$$

vary not only from one cell type to another, but also in relation to the particular experimental environment of the system under examination (1), our conclusions are expressed in terms of sharply defined experimental conditions and of a single tissue. The extension of these results to *in vivo* conditions is naturally a matter of great interest and importance. It can be done, however, only after the first objective has been attained, *i.e.*, a precise description of the chemical reactions occurring in a particular experimental system.

[1] Presented April 18, 1941, at the Symposium on Carbohydrate Metabolism at the Chicago meeting of the American Society of Biological Chemists. The original work was aided in part by grants from the John and Mary R. Markle Foundation and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

In the case of a suspension of minced pigeon breast-muscle in buffered saline at a pH of about 7.4, the whole of the experimental data concerning the oxidation of pyruvic acid has been generalized by Krebs and his coworkers into a scheme involving a cyclic chain of chemical transformations (2, 3, 4, 5). This scheme, the so-called citric acid cycle, represents a current summary of the pertinent data concerning pyruvate oxidation in muscle, and should be regarded as the basis for further experiment, rather than a proved analysis.

Consideration of the experimental evidence for this formulation begins with the so-called catalytic action of the dicarboxylic acids. When small quantities of fumaric, succinic, malic (6, 7, 8), α-ketoglutaric, and citric acids (2) are added to suspensions of muscle, there is an increase in the oxygen consumption of the tissue which is much greater than can be accounted for by the complete oxidation of the added compound.

If we add one of these dicarboxylic acids while the tissue is oxidizing pyruvic acid and measure the amount of pyruvate disappearing, we find that this increased oxygen consumption is associated with an increased utilization of pyruvic acid and that the amount of added dicarboxylic acid does not appreciably change. In other words, the dicarboxylic acid is promoting or catalyzing the oxidative reactions responsible for the respiration of the surviving tissue. It does this, we believe, by participating as a reactant in the series of chemical reactions constituting the respiration of the tissue. The concentration of the catalytic agent remains practically constant since there is a balance between the continuous synthesis of the catalyst and its rapid utilization and removal. For example, the rapid oxidation of citric acid to α-ketoglutaric acid by muscle can be quantitatively demonstrated in experiments in which one adds sodium arsenite to the tissue, this being a reagent which poisons those enzymes responsible for the further oxidation of α-ketoglutaric acid (2). Or, if we poison the tissue with malonic acid, a substance which inhibits the succinic dehydrogenase, *i.e.*, the enzyme which oxidizes succinic to fumaric acid, then we can demonstrate the quantitative and rapid conversion of added α-ketoglutaric acid to succinic acid (3). The subsequent oxidation of succinic acid to fumaric acid, the enzymic hydration of fumaric acid to malic acid, and the oxidation of the latter to oxaloacetic acid

are reactions known to occur in muscle tissue at rates and to an extent consonant with their being principal metabolic pathways. We can, therefore, write the following chain indicating the reactions undergone by citric acid in muscle:

Citrate ⟶ α-ketoglutarate ⟶ succinate ⟶

fumarate ⟶ malate ⟶ oxaloacetate.

The substances intermediate in this breakdown of citric acid, it will be noted, are the dicarboxylic catalysts mentioned before.

To make the story complete, it is necessary to demonstrate that the muscle is capable of the rapid synthesis of citric acid as well as of its destruction. A formation of citrate at the required rate can be demonstrated by adding oxaloacetic acid to muscle tissue in the absence of air to prevent the further oxidation of the citrate formed (2). This synthetic reaction requires, obviously, two additional carbon atoms. Originally, Krebs and Eggleston suggested that the source of these carbon atoms was an unspecified triose intermediate in carbohydrate metabolism. Subsequently, the identity of this triose with pyruvic acid was assumed. Although this assumption is probable and generally accepted, unequivocal proof that pyruvate is the source of these extra carbon atoms is still lacking. There is no question, however, that a rapid synthesis of citrate can be demonstrated under these conditions.

These reactions constitute the qualitative framework for the citric acid cycle. When the oxidation of carbohydrate in muscle reaches the stage of pyruvic acid, this combines with a small quantity of preformed oxaloacetic acid in the tissue and forms citric acid. The citrate is oxidized to α-ketoglutarate, α-ketoglutarate to succinate, this to fumarate, malate, and oxaloacetate, which condenses with another molecule of pyruvate. That is, a cyclic series of reactions occur whereby pyruvate is consumed and corresponding quantities of carbon dioxide and water are formed (fig. 1). The catalytic effect of the dicarboxylic acids becomes understandable, therefore, and it is clear that the experimental demonstration of the catalytic effect will depend: 1) upon the concentration of oxidizable substrate available (*i.e.*, pyruvate in the experiments under discussion); 2) the amount of di-

carboxylic acids and other necessary compounds already present in the tissue, *i.e.*, the "state" of the tissue; and 3) the quantity of acids added. It is evident that an occasional failure to obtain the catalytic effect with a particular suspension of tissue cannot be regarded as invalidating this conception of the role of the dicarboxylic acids.

These facts are a demonstration of the existence of the necessary enzyme systems in pigeon breast-muscle for carrying out the reactions

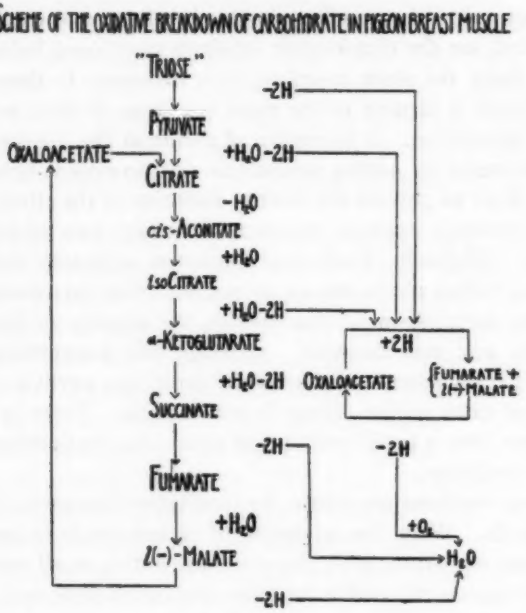

FIG. 1

indicated in figure 1. Evidence as to the extent to which these reactions account quantitatively for the total respiration of muscle when pyruvate is being oxidized must be considered next.

The most important experimental data concerned are summarized in the following equations.

1] In the presence of malonate:

$$\text{Fumarate} + \text{pyruvate} + 2O_2 \longrightarrow \text{succinate} + 3CO_2 + H_2O$$

2] In the absence of malonate:

$$\text{Succinate} + \tfrac{1}{2}O_2 \longrightarrow \text{fumarate} + H_2O$$

3] (1 + 2) $$\text{Pyruvate} + 2\tfrac{1}{2}O_2 \longrightarrow 3CO_2 + 2H_2O$$

When pyruvate is added to a suspension of pigeon breast-muscle, although the oxygen consumption increases only slightly, measurement of oxygen and pyruvate used and CO_2 produced indicates that the whole of the oxygen uptake of the tissue is utilized in the series of reactions comprising pyruvate oxidation. For each molecule of pyruvate disappearing, 2.5 mols of oxygen are used up and 3 mols of CO_2 are formed in quantitative agreement with equation 3 (3).

If we now add malonic acid to the system, both the oxygen consumption and the pyruvate utilization are almost completely inhibited—in the presence of 0.025 M malonate to more than 90 per cent. It is possible that the inhibiting effect of the malonate under these experimental conditions may not be entirely confined to the succinic dehydrogenase (9). However, this is certainly the principal effect and the assumption that the cessation of respiration is due to the blocking of succinate oxidation seems completely justified.

If we add fumaric as well as pyruvic acid to this malonate-poisoned tissue we find that oxygen is now taken up and the fumarate and pyruvate are both metabolized. We find for every mol of added fumarate, one mol of pyruvate and 2 mols of oxygen are consumed with the formation of 3 mols of carbon dioxide and 1 mol of succinate, *i.e.*, the stoichiometric relationships of equation 1 can be demonstrated by analysis for each of the substances concerned (3). This is exactly in accordance with the theory. It must be emphasized that this experiment is crucial in any explanation of pyruvate metabolism and of fumarate function since it demonstrates the existence of an oxidative route whereby succinate is formed from fumaric acid.

It remains to be shown then that oxaloacetate, citrate, and α-ketoglutarate, are intermediates in this process, *i.e.*, that the other reactions of the citric acid cycle are involved. Unequivocal quantitative data on this point are still lacking. If the scheme is correct, the intermediate stages between fumarate and pyruvate on one side and succinate on the other should all form succinate in the presence of malonate. Further, the yields of succinate must be no less and the rates of succinate formation no lower when these intermediates are

added than those found in the presence of pyruvate and fumarate. Krebs and Eggleston have published experiments (3) in which they find that the rates of oxygen consumption and the yields of succinate on adding the various intermediates listed in the scheme to the malonate-poisoned tissue are approximately of the expected order. The possibility remains of additional substances acting as intermediates, but the examination of a large number of compounds resulted in the tested substances not being oxidized or failing to yield succinate in the presence of malonate.

Baumann and Stare (10) have concluded from the inability of citrate to restore the original rate of respiration to malonate-poisoned tissue, that an intact citric acid cycle is not essential for the respiration of muscle. This conclusion is, however, open to criticism. The effects these workers describe were obtained in the absence of added substrate, *i.e.*, pyruvate. Assuming, however, that the carbohydrate substrate being oxidized was triose, the citric acid cycle of Krebs postulates that the oxidation of one mol of triose requires the withdrawal of six pairs of hydrogen atoms, and it follows that the total oxygen uptake of the muscle (respiring on triose in the absence of any added substrate) is a sum of the oxygen required for the conversion of: 1) triose to pyruvate; 2) pyruvate and oxaloacetate to citrate; 3) citrate to α-ketoglutarate; 4) α-ketoglutarate to succinate; 5) succinate to fumarate; and 6) malate to oxaloacetate. Since the total O_2 uptake is the sum of these six stages it follows that the rate of any individual step cannot be less than one-sixth of the total Q_{O_2}. If the rates of the various individual reactions are to be examined from the standpoint of their possible participation in the respiration of the tissue, then it becomes an indispensable criterion of such participation that the rate of any single step shall not be less than one-sixth the rate of respiration of the tissue.[2] When citrate, therefore, is added

[2] A demonstration of this proposition in general terms is as follows. If the oxygen uptake of m grams of tissue can be analyzed into a cycle of n consecutive reactions of the form:

$$1)\quad A + O_2 \longrightarrow B$$
$$2)\quad B + O_2 \longrightarrow C$$
$$3)\quad C + O_2 \longrightarrow D$$
$$\cdots\cdots\cdots\cdots$$
$$n)\quad N + O_2 \longrightarrow A$$

to malonate-poisoned muscle of which the normal Q_{O_2} is usually about 20 (11) the citric acid cycle requires that the rate of oxygen uptake

---

Then under conditions of a steady state:

$$dA/dt = 0 = dB/dt = dC/dt, \text{ etc.}$$

$$\text{and } R_A A = R_B B = R_C C = R_N N$$

$$\text{also } \frac{d\,(O_A)}{dt} = R_A A$$

$$\frac{d\,(O_B)}{dt} = R_B B$$

$$\frac{d\,(O_N)}{dt} = R_N N$$

Where:
- O_A, O_B, O_N represent the total quantity of O_2 consumed for reactions 1, 2, 3, etc.
- A, B, C = concentration of substances A, B, C, etc.
- R_A, R_B, etc. = number of mols of O_2 consumed per unit time per mol of A, B, etc. at the steady state.

Then: $$\frac{d\,(O_2 \text{ Total})}{dt} = R_A A + R_B B + R_C C + \cdots R_N N$$

$$\frac{d\,(O_2 \text{ Total})}{dt} = n(R_A A) = n(R_B B), \text{ etc.}$$

And: $R_A A$ = rate of oxygen uptake per m grams of tissue per unit time for any step $= 1/n \cdot \dfrac{d(O_2 \text{ Total})}{dt}$

Under conditions of a steady state, these equations require that each step of the cycle consumes an equal quantity of oxygen although the rate of oxygen uptake per *mol* of intermediate may vary. If we are to isolate and examine individual reactions from the standpoint of their possible participation in a postulated cycle of the type outlined, it follows that the rate of oxygen uptake for the individual step cannot be less than $1/n$ of the total oxygen uptake. A comparison of this sort requires, first, that the concentration of enzyme systems and reactants participating in the isolated step should be the same as that present in the original experimental system, and second, that the possible inhibiting effect of reaction products accumulating under the conditions of the isolated experiment should be eliminated as the cause of the rate being below the minimum level. On the other hand, if the isolated reaction proceeds with a velocity greater than $1/n$ of the total oxygen consumption, the reaction can still be considered a possible intermediate since in the intact and total system the concentration of enzyme systems necessary for the individual reactions may be greater than the available substrate. Under

for this particular reaction (involving two oxidative steps, the oxidation of citrate to α-ketoglutarate and of α-ketoglutarate to succinic acid) shall not be less than $\frac{20}{3}$, or 6.6. Under the experimental conditions of Baumann and Stare the total Q_{O_2} of the tissue is about 8.1 and the Q_{O_2} for citrate in malonate-poisoned muscle is about 3.6, *i.e.*, greater than $\frac{8.1}{3}$. Rather than demonstrating a non-participation of citrate in the respiratory cycle of muscle, this indicates that the rapidity of citric acid removal is within the required limits. The results of Baumann and Stare, therefore, are neither in contradiction of the citric acid cycle nor, for that matter, do they offer support for the hypothesis.

Further evidence for the validity of the citric acid cycle is offered by experiments in which large quantities of pyruvate are added to the malonate-poisoned tissue (3). Since pyruvate is preferentially oxidized, an excess of pyruvate would be expected to cause an accumulation of intermediates. When fumarate with an excess of pyruvate is added to malonate-poisoned muscle, α-ketoglutarate accumulates in quantities of about 50 per cent of the theoretical and citrate in quantities of about 15 per cent of that theoretically required. In the absence of specific methods for interrupting the cycle at each of the intermediate steps, these incomplete yields cannot be regarded as the basis for any objection to the theory.

As we have seen, the oxidation of 1 mol of pyruvate in terms of the scheme consists of a series of five oxidative steps. Krebs and his coworkers have concluded that in two of the steps—the oxidative condensation of pyruvate and oxaloacetate to citrate and the oxidation of citrate to α-ketoglutarate—the oxaloacetate-malate system acts as a hydrogen carrier. This is indicated schematically in fig. 1. The experimental basis for this conclusion lies in the demonstration of the following reactions when the reagents involved are added to muscle tissue under anerobic conditions.

1] 2 oxaloacetate + pyruvate ⟶ citrate + CO_2 + malate

2] oxalacetate + citrate ⟶ α-ketoglutarate + CO_2 + malate.

---

such circumstances, the isolation of the system and the addition of substrate in excess would give a rate of oxygen uptake greater than $1/n$ of the total oxygen consumption.

The observed changes are of the indicated magnitude and occur at the necessary rates as calculated in terms of the aerobic process. However, the demonstration that these reactions occur at the required rate in the presence of oxaloacetate does not necessitate oxaloacetic acid being the normal link between the substrate in question and oxygen. As Potter has pointed out in a recent review (12) the presence of the diphosphopyridine and triphosphopyridine nucleotides, and of possible unknown intermediates in muscle tissue as hydrogen carriers common to reversibly oxidized metabolites of the kind being discussed here can explain anerobic reactions of the observed type. The conclusion is not that the oxaloacetate-malate system does not serve as a hydrogen carrier in the citric acid cycle, but that there is, as yet, no acceptable evidence of such a function.

This represents in brief outline the pertinent facts about the utilization of pyruvate by pigeon breast-muscle, the tissue in which the most exhaustive study of these reactions has been made. Similar conclusions in regard to pig heart-muscle have been reached by Smyth (13).

In pigeon liver, the available data indicate the necessity for modifying the scheme presented for muscle. Pyruvic acid is rapidly oxidized by minced pigeon liver suspended in a buffered saline medium. The ratio of oxygen consumed to pyruvate disappearing is about 0.6; the R.Q., 1.4, *i.e.*, the oxidation of pyruvate is incomplete in contrast to muscle where pyruvic acid is completely oxidized to carbon dioxide and water. With liver suspensions, concentrations of malonic acid which cause a complete inhibition of pyruvate oxidation in muscle are without effect (14).

There can be no question that the succinic dehydrogenase system is present in liver, and that the concentrations of malonate we are using are sufficient to poison it, so that the conclusion is inescapable that neither the utilization of pyruvic acid nor the uptake of oxygen by minced liver, require the activity of the succinic dehydrogenase. It is quite clear, then, that the utilization of pyruvic acid by minced pigeon liver, under the conditions of these experiments, is unlike the process that occurs in muscle.

Two compounds which must be considered as products of the incomplete oxidation of pyruvic acid were found to accumulate in pi-

geon liver suspensions under the conditions of these experiments; namely, acetoacetic acid and α-ketoglutaric acid. The formation of acetoacetic acid from pyruvic acid in liver has been previously described (15, 16, 17). The synthesis of α-ketoglutaric acid from pyruvate by pigeon liver is a reaction which requires some further discussion. The rapidity and magnitude of the process may be discerned from Table I. After 40 minutes, the quantity of α-ketoglutarate found remains approximately constant. This may be interpreted as a balance between synthetic and oxidative reactions inasmuch as α-ketoglutaric acid is rapidly oxidized by the minced liver. The rapid oxidation of α-ketoglutarate prevents any estimate of the extent to

TABLE I

Synthesis of α-Ketoglutaric Acid in Pigeon Liver

4 ml. 1–10 suspension of liver in Ca-free phosphate saline, pH 7.4; initial pyruvate concentration, 0.03 M; temp., 40°C; gas phase, O_2.

TIME	O_2 USED	PYRUVATE USED	α-KETOGLUTARIC ACID FOUND
min.	*μl*	*μl*	*μl*
20	800	1560	227
40	1590	2420	284
80	2925	2653	287
120	3765	2675	203

1 mole = 22,400,000 μl.

which α-ketoglutaric acid formation accounts for the pyruvic acid which has disappeared. It is possible, however, to prevent the further oxidation of succinate, the initial product of the oxidation of α-ketoglutaric acid, by adding malonic acid to inhibit the succinic dehydrogenase. In such circumstances the measurement of the amount of α-ketoglutarate + succinate formed should serve as an indication of the quantitative importance of α-ketoglutarate synthesis in pyruvate oxidation by pigeon liver (Table II). While the malonate has no effect on the quantities of pyruvate or oxygen consumed, increased amounts of both acetoacetate and α-ketoglutarate + succinate are formed. On the assumption that each molecule of acetoacetate, or of α-ketoglutarate + succinate, requires 2 molecules of pyruvate for its formation, the bulk of the pyruvate disappearing can be accounted

for in terms of these metabolites. In the experiment cited, the α-ketoglutarate and succinate account for about 60 per cent of the added pyruvate, and the acetoacetate for another 25 per cent. In view of the fact that the rate of pyruvate utilization by liver is not affected by malonate, it is probable that the two reactions (α-ketoglutarate and acetoacetate synthesis) which can be demonstrated as accounting for the bulk of pyruvate used by malonate-poisoned tissue, are of similar quantitative importance in the oxidation of pyruvate by liver in the absence of malonic acid. It follows that the observed differences between pyruvate utilization in muscle and in liver are due largely to the presence of these additional reactions in liver. While the muscle

TABLE II

Formation of Acetoacetic Acid and α-Ketoglutaric Acid in the Presence of Malonate

4 ml. 1–10 liver suspension in Ca-free phosphate saline; pH 7.4; pyruvate (initial concentration, 0.03 M) added from side-arm after 10 minutes, malonate added directly to tissue; temp., 40°C; gas phase, O_2; experimental period, 30 minutes.

ADDITION	O_2 USED	PYRUVATE USED	ACETOACETIC ACID FORMED	α-KETOGLUTARIC AND SUCCINIC ACIDS FOUND
	μl	μl	μl	μl
0.025 M malonate	580	—	34	164
0.03 M pyruvate	1097	1605	38	233
0.025 M malonate, 0.03 M pyruvate	952	1640	233	518

is incapable of forming appreciable quantities of the C_4 dicarboxylic compounds used in its metabolism, the liver is able to effect such a synthesis, since the α-ketoglutaric acid which accumulates under the particular experimental conditions here used, can be further oxidized to succinate, fumarate, and oxaloacetate. The general metabolic significance of the reaction may be that the liver serves as a source of the dicarboxylic acids (possibly transported in some other form) for those tissues incapable of their direct formation.

The mechanism by which α-ketoglutarate is formed from pyruvate under the conditions of these experiments is of considerable interest. Krebs and Eggleston (18) find that in the presence of bicarbonate and CO_2 the rate of pyruvate utilization in liver is 37 to 94 per cent above that of the controls. There is also a large increase in the formation

of α-ketoglutarate (115 to 170 per cent), of citrate (160 to 560 per cent), and of fumarate + malate (125 to 400 per cent). From the fact that the same substances, *i.e.*, α-ketoglutarate, fumarate, malate, succinate, and citrate are formed from added pyruvate as from added oxaloacetate in both aerobic and anerobic conditions, and from the specific effect of carbon dioxide mentioned above, Krebs and Eggleston have concluded that the synthesis of α-ketoglutarate from pyruvate involves first the condensation of pyruvate with carbon dioxide to form oxaloacetic acid, this combining with another mol of pyruvate to form citrate, and this, in turn, being oxidized to α-ketoglutarate. A similar carboxylation of pyruvate to oxaloacetate had been previously proposed by Wood and Werkman (19) as an intermediate reaction in the metabolism of the propionic acid bacteria and the occurrence of the other reactions in pigeon liver has been previously demonstrated (14).

In experiments published independently and simultaneously with those of Krebs and Eggleston, Slotin and I have been able to demonstrate the participation of carbon dioxide in α-ketoglutarate synthesis by the use of radioactive C_{11} as a tracer substance (20). By adding pyruvic acid to a suspension of minced pigeon liver in a bicarbonate-buffered saline containing radioactive bicarbonate and isolating the synthesized α-ketoglutarate as the dinitrophenylhydrazone, the intense radioactivity of the compound unquestionably demonstrated that carbon dioxide participates in the synthetic reactions involved. The quantity of radioactivity appearing in the compound makes it improbable that we are dealing with a non-specific exchange reaction. Quantitative data are given in Table III. Of the original activity about 5 to 10 per cent can be accounted for in the synthesized α-ketoglutarate. Continuous dilution of the radioactive carbonate by metabolic carbon dioxide complicates any quantitative interpretation of the data in terms of a specific mechanism, but from the ratio of α-ketoglutarate activity per mgm. carbon to medium activity per mgm. carbon, and from the results of the experiments next described, it is probable that one carbon atom per molecule of α-ketoglutarate is obtained from the carbon dioxide of the medium.

The synthesis of α-ketoglutarate by way of the reactions of the citric acid cycle would require that half the synthesized α-ketoglu-

tarate molecules would contain radioactivity in the carboxyl group alpha to the ketonic oxygen and the other half carry radioactive carbon in the omega carboxyl (fig. 2). Oxidation of the α-ketoglutarate synthesized according to this scheme should yield succinic acid with half the radioactivity of the α-ketoglutarate. However, when the radioactive α-ketoglutarate obtained from our first experiments is oxidized with acid permanganate at room temperature, *i.e.*, under conditions which quantitatively convert α-ketoglutarate to succinate, we find that the succinate formed is entirely inactive and that the entire radioactivity can be recovered as CO_2. These results cannot be reconciled

TABLE III

Synthesis of α-Ketoglutaric Acid in Radioactive-Bicarbonate Medium

50 ml. Ca-free bicarbonate saline, pH 7.4, containing radioactive C_{11}; 1.7 cc. M/10 malonate; 10 cc. M/5 pyruvate; malonate added directly to tissue; pyruvate added after 10 minutes; temp., 40°C; gas phase, 5% CO_2, 95% O_2; experimental period, 40 min.

EXPERIMENT NUMBER	α-KETOGLUTARATE SYNTHESIZED	ACTIVITY OF α-KETOGLUTARATE PER MGM. C	ACTIVITY* OF MEDIUM PER MGM. C	RATIO: ACTIVITY OF α-KETOGLUTARATE / TOTAL ACTIVITY	RATIO: ACTIVITY OF α-KETOGLUTARATE PER MGM. C / ACTIVITY MEDIUM PER MGM. C*
	mgm.	*Div./sec.*†		*per cent*	
1	57.8	0.007	0.0245	10.8	3.5
2	55.0	0.171	1.17	4.8	6.9
3	27.5	0.362	1.13	6.1	3.1

* Activity of carbon dioxide released from medium by acidification.

† Activities in divisions per second (Lauritsen electroscope) corrected for decay so that the values are comparable.

with the suggested mechanism, nor, because of its symmetrical nature, can citric acid be an intermediate in the process. It is possible that carbon dioxide can be assimilated by means of a carboxylation of pyruvic acid to oxaloacetic acid, but this substance is not converted into α-ketoglutaric acid through the reactions of the citric acid cycle. The available data are still insufficient to suggest an alternate mechanism. It should be emphasized, however, that these data are concerned with α-ketoglutarate formation in liver and the demonstration that this synthesis cannot involve the citric acid cycle in its present form does not invalidate the evidence for the occurrence of the cycle in muscle.

The assimilation of carbon dioxide by the tissues of higher animals is a reaction of considerable interest and novelty. In the single tissue studied by us, the reaction is of appreciable magnitude. In the intact animal, Hastings and his collaborators have reported that radioactive glycogen is formed when radioactive sodium bicarbonate is injected (21). It seems possible that we shall be forced to modify

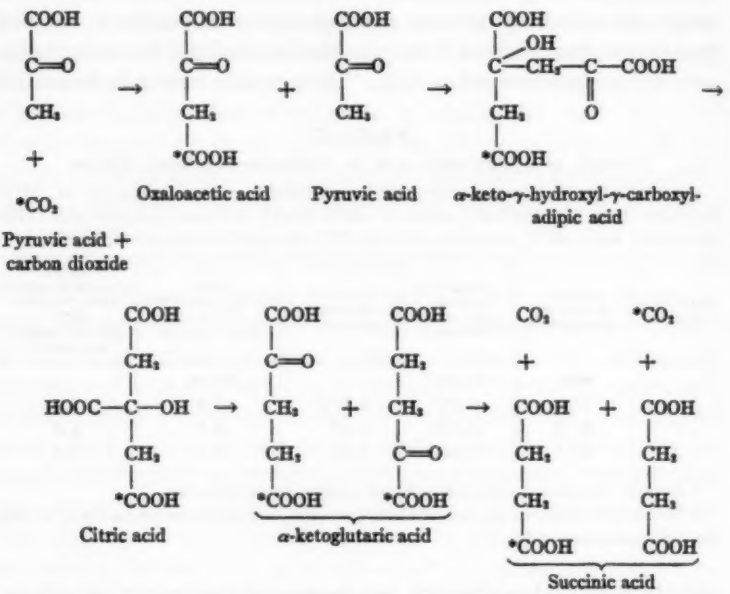

FIG. 2. Scheme for the synthesis of α-ketoglutaric and succinic acids from carbon dioxide and pyruvic acid by way of the citric acid cycle. The * represents radioactive carbon atoms.

the view that the metabolic release of CO_2 is effected by reactions proceeding exclusively in one direction.

BIBLIOGRAPHY

(1) BARRÓN, E. S. G., AND LYMAN, C. M.: *J. Biol. Chem.*, **127**: 143, 1939.
(2) KREBS, H. A., AND JOHNSON, W. A.: *Enzymologia*, **4**: 148, 1937.
(3) KREBS, H. A., AND EGGLESTON, L. V.: *Biochem. J.*, **34**: 442, 1940.
(4) KREBS, H. A.: *Biochem. J.*, **34**: 460, 1940.

(5) KREBS, H. A.: *Biochem. J.*, **34:** 775, 1940.
(6) GOZSY, B., AND SZENT-GYÖRGYI, A.: *Z. physiol. Chem.*, **224:** 1, 1934.
(7) ANNAU, E., BANGA, I., GOZSY, B., ST. HUSZAK, LAKI, K., STRAUB, B., AND SZENT-GYÖRGYI, A.: *Z. physiol. Chem.*, **236:** 1, 1935.
(8) STARE, F. J., AND BAUMANN, C. A.: *Proc. Roy. Soc.*, B, **121:** 338, 1936.
(9) WEIL-MALHERBE, H.: *Biochem. J.*, **31:** 299, 1937.
(10) BAUMANN, C., AND STARE, F., *J. Biol. Chem.*, **183:** 13, 1940.
(11) KREBS, H. A., AND EGGLESTON, L. V.: *Biochem. J.*, **32:** 2125, 1938.
(12) POTTER, V. R.: *Medicine*, **19:** 441, 1940.
(13) SMYTH, D. H.: *Biochem. J.*, **34:** 1046, 1940.
(14) EVANS, E. A., JR.: *Biochem. J.*, **34:** 829, 1940.
(15) EMBDEN, G., AND OPPENHEIMER, C.: *Biochem. Z.*, **55:** 335, 1913.
(16) ANNAU, E.: *Z. physiol. Chem.*, **224:** 141, 1934.
(17) EDSON, N. L.: *Biochem. J.*, **29:** 2082, 1935.
(18) KREBS, H. A., AND EGGLESTON, L. V.: *Biochem. J.*, **34:** 1383, 1940.
(19) WOOD, H. G., AND WERKMAN, C. H.: *Biochem. J.*, **34:** 129, 1940.
(20) EVANS, E. A., JR., AND SLOTIN, L.: *J. Biol. Chem.*, **136:** 301, 1940.
(21) VENNESLAND, B., SOLOMON, A. K., KLEMPERER, F. W., AND HASTINGS, A. B.: Reported at the Chicago meeting of the American Society of Biological Chemists, April, 1941.

ELECTROENCEPHALOGRAPHIC CHANGES ASSOCIATED WITH DIFFERENT FORMS OF EXPERIMENTALLY PRODUCED INCREASED INTRACRANIAL PRESSURE

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This paper reports on experiments investigating the relationship of various forms of increased intracranial pressure to large slow waves in the electroencephalogram. Previous studies have found large slow waves associated in some cases with increased intracranial pressure. However, no correlation has been found between the height or duration of the pressure and the presence of these waves (1, 2, 3). This lack of correlation suggests that the large slow waves do not result directly from increased intracranial pressure, and that some additional cause must be sought to explain their presence.

It has been observed that the large slow waves disappear following reduction of intracranial pressure by ventricular puncture (4). Williams has temporarily eliminated the large slow waves associated with increased intracranial pressure, by the intravenous injection of hypertonic salt solution (3). The elimination of large slow waves occurs with only a small reduction in the intracranial pressure, as measured by lumbar puncture. He concluded that the large slow waves were caused by edema associated with raised intracranial pressure, "probably an edema of the nerve fibres of the white matter of the brain."

Certain inconsistencies seem apparent in the above data. If the large slow waves were a direct result of increased pressure they should be present in all cases, and should show some correlation with the height or duration of the pressure. Since they do not, one may conclude that they are not the direct result of pressure. It would also seem unlikely that large slow waves are caused by brain edema since

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they disappear quickly following reduction of pressure by ventricular tap.

There is a possibility that the large slow waves are caused, not by all forms of increased intracranial pressure, but by some specific form. Two forms may be differentiated:

(1) *Internal hydrocephalus.* The channels through which cerebrospinal fluid normally circulates, are blocked. The fluid accumulates under increasing pressure in the ventricles. The increased volume of ventricular fluid acts as an expanding force enlarging the ventricles and ironing out the cortical convolutions.

(2) *A disturbance in the normal equilibrium between production and absorption of cerebrospinal fluid.* Normally fluid escapes from a capillary at its arterial end. Here the hydrostatic pressure is greater than the colloid osmotic pressure of the blood. At the venous end of the capillary, the colloid osmotic pressure predominates and fluid reenters the blood vessel (5). If this balance is disturbed by venous hypertension or blood dilution, the forces promoting absorption of the fluid are diminished. This results in increased pressure of cerebrospinal fluid which, in turn, promotes absorption. These two opposing forces neutralize each other and a new equilibrium is established at a higher pressure. The net result is an increase in the pressure of the cerebrospinal fluid, associated with brain edema. Since there is no increase in the volume of cerebrospinal fluid, no major deformity of the brain develops. This type of disturbance will be referred to as *generalized increased intracranial pressure* in order to distinguish it from *increased intraventricular pressure.*

In earlier observations on the relation of intracranial pressure to large slow waves in the electroencephalogram, no distinction has been made between increased intraventricular pressure and generalized increased pressure. Observation of clinical cases of active internal hydrocephalus has shown that large slow waves are prominent in the electroencephalogram (2). It may be that the difference in these forms of increased pressure accounts for the presence or absence of the large slow waves.

To test this hypothesis it was decided to investigate the brain waves of both these forms of increased intracranial pressure in experimental animals. Experimental intraventricular pressure, acute generalized

pressure and chronic generalized pressure are considered under separate headings and the results are discussed in a final section.

INCREASED INTRAVENTRICULAR PRESSURE—HYDROCEPHALUS

Methods

Kittens were used as experimental animals. Adult cats were unsatisfactory because their skulls were too rigid.

The technique used to produce hydrocephalus was that devised by Weed (6). Under very light Nembutal anaesthesia a cisternal tap was performed; 0.3 cc. of cerebrospinal fluid was withdrawn and replaced by 0.5 cc. of a 10 per cent suspension of lampblack. This occluded the pathways for circulation of the cerebrospinal fluid and produced hydrocephalus.

The mortality rate of the procedure was high. The work involved in taking electroencephalograms on all animals prior to operation was not considered justified, since many died before developing hydrocephalus. Control records, therefore, were made on a separate group of kittens. Electroencephalograms were taken after the injection of lampblack and during the rest of the kitten's life at irregular intervals varying from one day to a month apart. Records were taken through the intact skull by means of four solder electrodes which were fixed to the scalp with adhesive tape, contact being made with electrode jelly. There were two frontal and two occipital electrodes. Each electrode was hooked up push-pull to its neighbor, resulting in a closed circle and four bipolar channels: right and left fronto-occipital, trans-frontal and trans-occipital.

The records were taken on the unanaesthetized kitten when at rest but not asleep. An assistant remained in the cage at some distance from the kittens to see that the animals remained awake during the recording. When the animals died or were killed, the brains were fixed in formalin, cut, photographed and sectioned in order to show the extent of hydrocephalus.

Results

Control Records. Electroencephalographic records were made on three normal unanaesthetized kittens. The results, shown in Fig. I, resemble the normal records of adult cats.

Experimental. Experiments were conducted and records are presented on six young kittens. In this group the course and extent of the hydrocephalus varied considerably, as is shown by the photographs of the brains taken at autopsy (Fig. II). Figs. III to VIII are ten inch samples of the electroencephalographic records taken during the development of hydrocephalus. Comments on these records are grouped together for comparison in Table I. The changes in the

records fell into three groups which coincided with three stages in the development of hydrocephalus.

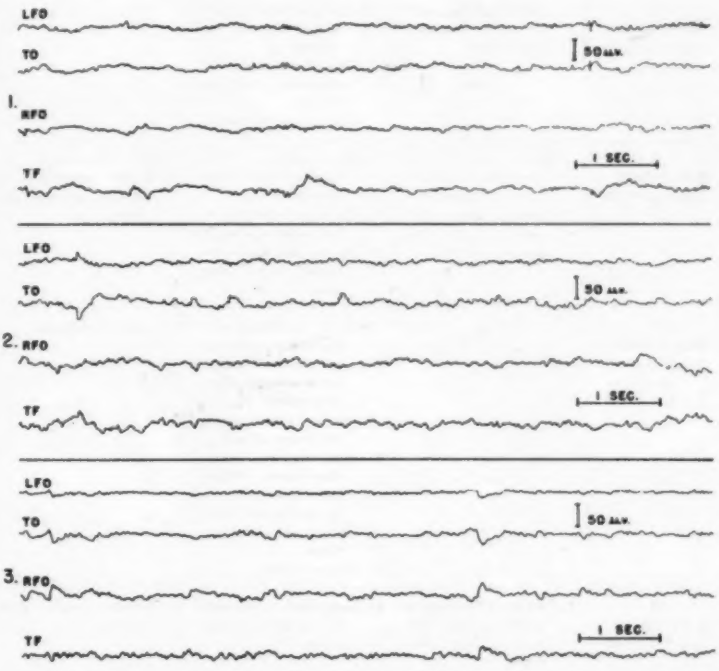

FIG. I. Normal electroencephalograms of 5 day old kittens. These records provide control normals for comparison with the records of hydrocephalic kittens.

In these and the succeeding records the following abbreviations are used to identify the position of the two electrodes which form each channel: L.F.O., left fronto-occipital; T.O., transoccipital (left to right); R.F.O., right fronto-occipital; T.F., transfrontal (right to left).

Records of the kittens were taken without anaesthesia. The adult cats received intraperitoneal Nembutal (1.0–1.5 cc.) sufficient to produce light anaesthesia.

The records first showed waves of a frequency of 15–20 per second and an amplitude of 25 microvolts. These may be characterized as "fast spiky" waves. This first stage existed from about the second to the ninth day following the injection of lampblack, and ended

TABLE I

Electroencephalographic studies on six kittens during the development of hydrocephalus

ANIMAL	FIRST STAGE OF PRESSURE	HYDROCEPHALUS	
		Moderate	Advanced
A Born 2-12-40 Inject. 2-16-40	1. Generalised fast activity with occasional slow wave	5. Clinical hydrocephalus: high frequency, low voltage. Ventricular tap: low frequency, moderate voltage. Clinical improvement.	
B Born 3-19-40 Inject. 3-29-40	5. Early clinical hydrocephalus. E.E.G.: fast, spiky waves. 7. Frontal: fast, spiky wave. Occipital: smooth, flat line.	11. Fast activity. Few large waves from frontal lead. 14. Fast spiky waves with occasional slow waves. 15. Pattern flat with occasional large slow wave.	22. Large slow waves. 24. Animal moribund. E.E.G. smooth, flat lines.
C Born 3-10-40 Inject. 3-29-40		5. Very hydrocephalic. Spiky waves of moderate voltage imposed on occasional large, slow waves. 7. Similar to last record, but lower voltage. 11. Left temporal: large, slow waves. Other leads show evidence of accidental subarachnoid haemorrhage. 15. Injury clearing.	30. Large, slow waves.
D Born 1-10-40 Inject. 1-18-40	1. Fast activity except in posterior lead. Occasional large, slow wave. 4. Fast, spiky activity. 14. Fontanelles open. Fast, spiky waves. Animal reinjected.	15. Large slow waves with fast activity superimposed.	16. Typical large, slow waves.
E Born 1-10-40 Inject. 2-26-40		29. Chronic hydrocephalus. Few, fast waves. Many large, slow waves.	39. Animal drags head. Large, slow waves. 47. Large, slow waves reduced when animal fastens attention on environment.
F Born 2-12-40 Inject. 2-16-40	6. Smooth, flat lines. Increased activity following ventricular tap.	8. Slow waves. Increased activity following ventricular tap.	9. Slow waves. Increased activity following tap.

Numbers indicate days on which records were taken.

The record for each animal is divided into three periods: (1) the period extending from the injection of lampblack to the onset of signs of hydrocephalus, (2) a period of moderate hydrocephalus, and (3) a period of advanced hydrocephalus. The figure preceding each comment indicates the number of days between the injection of lampblack and the electroencephalographic record.

FIG. II. A to F are photographs of the brain of the six hydrocephalic kittens reported. G is a photograph of the brain of an adult cat which had chronically increased intracranial pressure and brain edema of 32 days' duration. Records from this cat are shown in Fig. XIII.

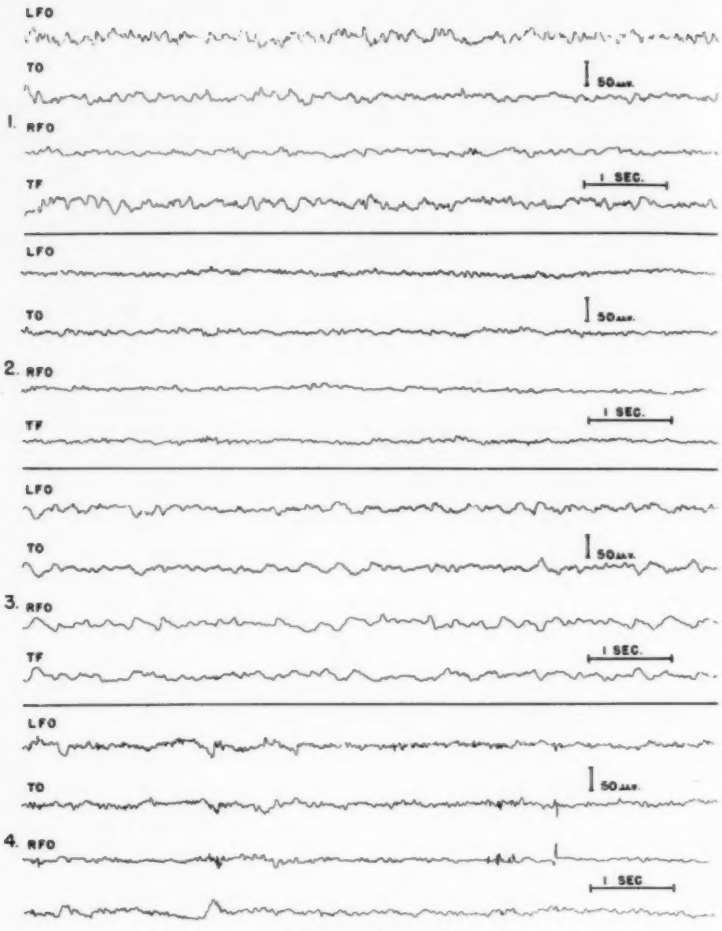

FIG. III. KITTEN A

The experimental period for this kitten covers 5 days. The electroencephalogram illustrates the change in the early acute stages of increased intraventricular pressure. Record 1 was taken 6 hours after injection of lampblack into the cisterna magna. It acts as a control for the effect of pressure. Records 2–4 were all taken on the fifth experimental day. Record 2 shows a reduction in voltage and an increase in frequency of the brain waves. During this record the animal was *in extremis*. Record 3 was taken on the same day, one and a half minutes after 1 cc. of cerebrospinal fluid had been removed by a ventricular tap. The kitten showed noticeable improvement and the brain waves are more nearly normal. Record 4 was taken five minutes after the withdrawal of fluid. Note the return of the high frequency, low amplitude waves.

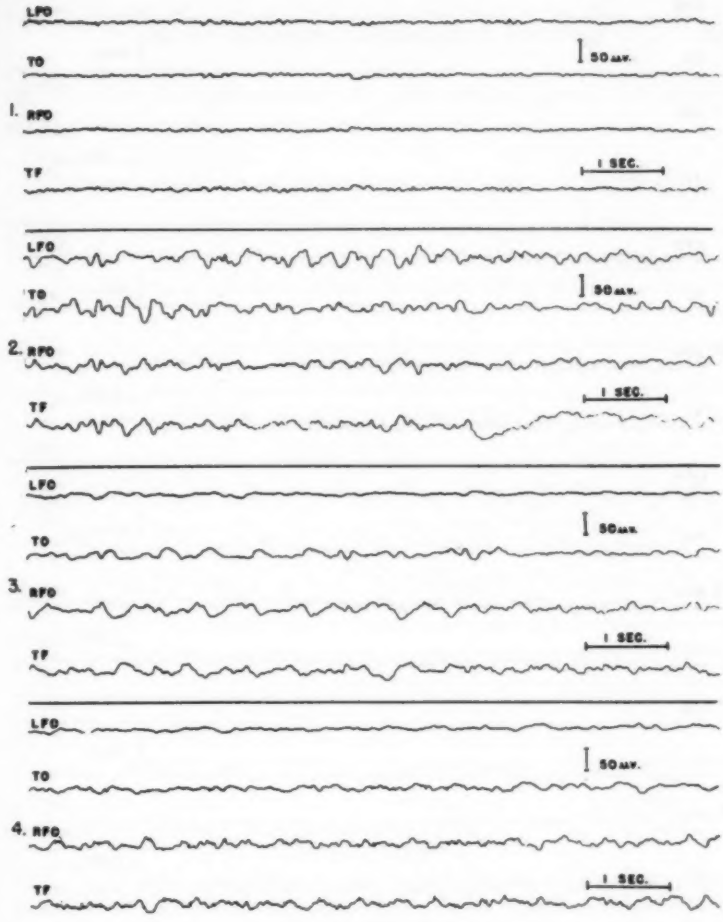

FIG. IV. KITTEN F

The experimental period for this kitten covers 9 days. This case illustrates an acute onset of increased intraventricular pressure which developed into hydrocephalus. Record 1 was taken 6 days after injection when the kitten was nearly moribund. The brain waves reflect this condition. Record 2 was taken on the eighth day. The kitten was in better condition and showed signs of hydrocephalus. Note the presence of large slow waves. Records 3 and 4 were taken on the ninth day. Slow waves are present in the third record. Following this record 2 cc. of cerebrospinal fluid were withdrawn by ventricular tap and record 4 was taken. The slow waves present before the reduction of pressure are much less conspicuous in this record.

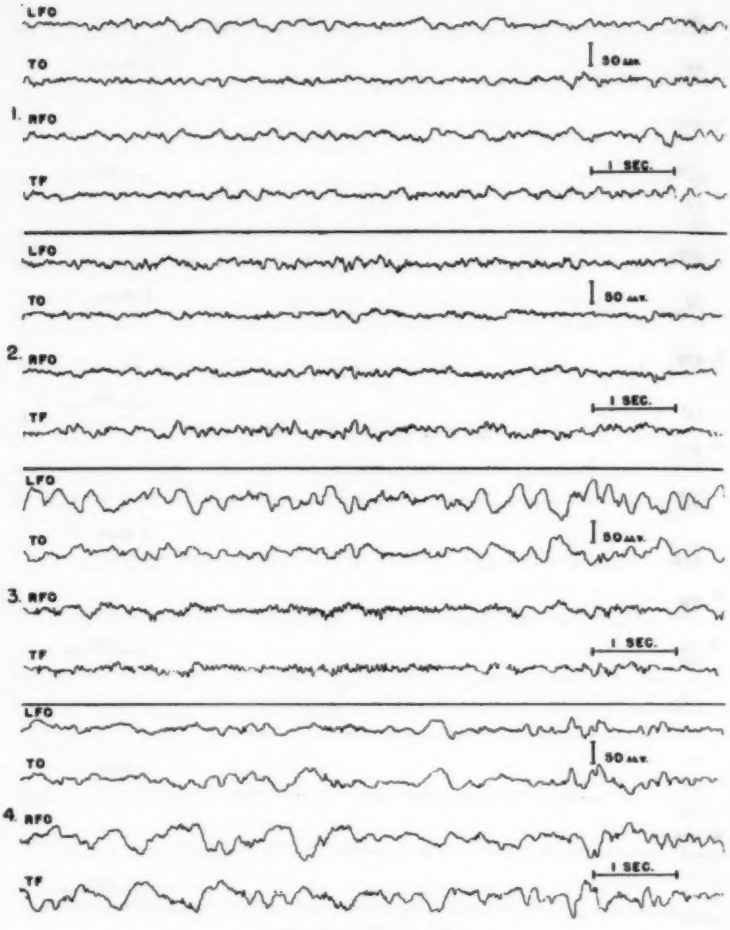

FIG. V. KITTEN D

The experimental period for this kitten covers sixteen days. These records illustrate all the changes in the pattern from normal to the large slow waves of active hydrocephalus. Record 1 was taken prior to injection of lampblack, when the kitten was 8 days old. Record 2 was made on the fourth day, and shows the fast spiky waves which occur as a transitional stage from the normal to the pattern of hydrocephalus. These waves represent the stage before signs of hydrocephalus have occurred. Record 3, taken on the fifteenth day, shows a combination of large slow waves on the left fronto-occipital and the trans-occipital channels, and fast spiky waves from the right fronto-occipital and trans-frontal channels. The fourth record, taken on the sixteenth day, shows large slow waves present in all channels.

shortly after the appearance of the very earliest signs of hydrocephalus.

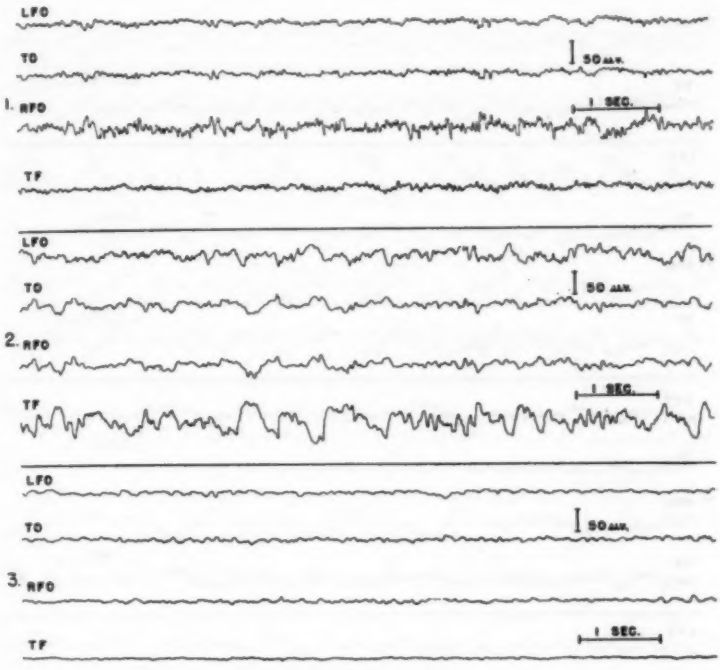

FIG. VI. KITTEN B

This kitten lived a total of 24 days after the intracisternal injection of lampblack. Record 1 was taken on the fifth day. Note the fast spiky waves present in all leads. Record 2 was taken on the seventeenth day. The signs of hydrocephalus were marked. The pattern shows the large slow waves which have characterized all those cases of sustained intraventricular pressure resulting in hydrocephalus. Record 3, taken on the twenty-fourth day, illustrates the pattern of the brain waves of a moribund animal.

In the second stage the electroencephalogram showed waves of about 3–5 per second and 150 microvolts. At this stage fast spiky waves were still evident, and large slow waves appeared intermittently. This second stage covered the period of the ninth to the fifteenth

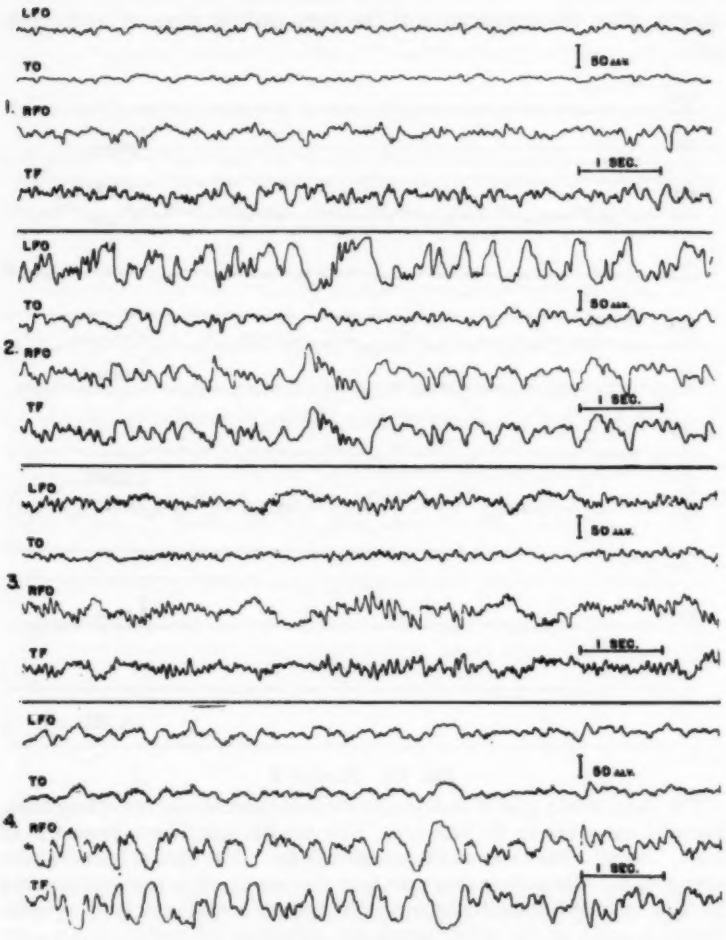

FIG. VII. KITTEN C

This animal showed the most marked hydrocephalus of the group. Record 1, taken on the fifth day, shows fast spiky waves superimposed on an occasional large slow wave. This pattern in brain waves is correlated with the transition from increased intraventricular pressure to hydrocephalus. The kitten showed signs of moderate hydrocephalus at this time. Records 2–4 were made on the 30th day. Large slow waves are present in records 2 and 4. The third record shows the variation which may occur in a single run. During this record the kitten's attention was held by the activity around him.

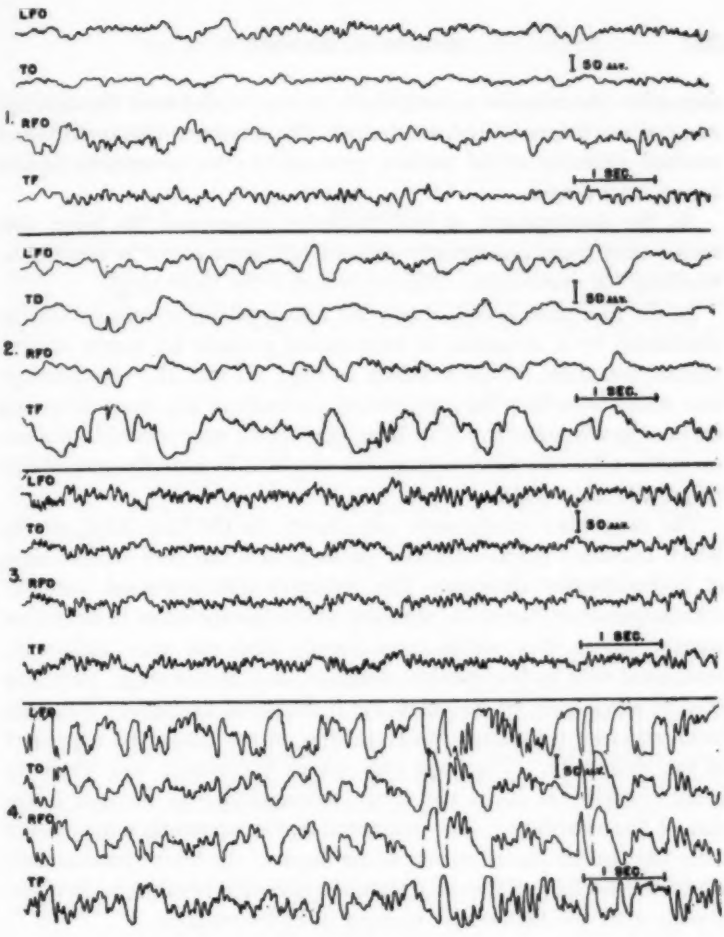

FIG. VIII. KITTEN E

This kitten lived the longest of the group and illustrates the gradual development of hydrocephalus. The kitten, when sacrificed, was almost two months old and had been under observation for 47 days. Record 1 was taken on the twenty-ninth day. Up to this time the development of hydrocephalus had been so gradual that it was uncertain whether the injection had been successful. Note that the brain waves at this time show the presence of occasional large slow waves. Record 2, taken on the thirty-ninth day, showed marked large slow waves of about 2 per second and over 300 m.v. Record 3 was taken on the 47th day. The kitten had his attention fixed on activity around him. This results in a decrease in amplitude and an increase in frequency of the brain waves. Record 4 was also taken on the forty-seventh day. The animal's attention was no longer held and the waves were so large that the machine became blocked in trying to record the voltage.

days after the injection of lampblack, and coincided with the development of overt signs of hydrocephalus. The kittens at this time showed marked widening of the sutures, protruding eyes, prominent frontal bosses, and ataxia.

As the development of hydrocephalus progressed the large slow waves were more consistently present and were larger in amplitude, reaching 300 microvolts. This represented the third stage.

In the first and second stages the abnormal brain waves could be eliminated by a reduction in intracranial pressure by means of ventricular puncture. This is shown in Figs. III and IV. No attempt was made to reduce the intracranial pressure in the most advanced cases of hydrocephalus. The large slow waves were reduced for a few moments when the kitten's attention was held by activity around him (Figs. VII and VIII).

The large slow waves were not present in the first stage, during which increased intraventricular pressure and the very earliest signs of hydrocephalus develop. This indicates that increased intraventricular pressure cannot be regarded as the specific cause of large slow waves. Large slow waves occur shortly after the brain deformity, associated with hydrocephalus, develops; and at this stage, they may still be eliminated by reducing the intracranial pressure. Since the brain deformity remains, the large slow waves cannot be a product of this deformity. The large slow waves are present only when the brain is under the stress and strain accompanying an increase in the size of the ventricles. The abnormal cortical potentials may result from the tension placed on the white fibres of the brain; tension sufficient to produce abnormal cortical potentials evidently develops shortly after the appearance of signs of hydrocephalus.

Large slow waves have been observed in the records of a small number of clinical cases of active hydrocephalus. An electroencephalogram from such a case is presented in Fig. IX.

These observations lead to the conclusion that *active internal hydrocephalus* is *one cause of large slow waves in the electroencephalogram.*

ACUTE INCREASED INTRACRANIAL PRESSURE WITHOUT HYDROCEPHALUS

Observation of clinical material shows that there are cases of increased intracranial pressure in which large slow waves are not present

in the electroencephalogram (2, 3). The following experiments were undertaken to investigate the brain waves of a form of markedly increased pressure of short duration, without hydrocephalus.

Methods

Adult cats were used as experimental animals because they were sturdier and easier to obtain. There were no forces tending to enlarge the ventricles and thereby distort the brain, so an adult, in which the skull sutures were closed, could be used. Control records presented earlier show that the change in age of the ex-

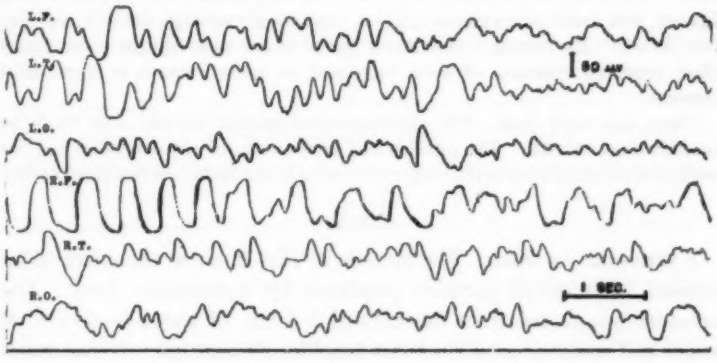

FIG. IX. Electroencephalogram of a one year old child (W) with recent acute hydrocephalus. Autopsy disclosed marked hydrocephalus caused by a cystic haemangioma of the cerebellum. Abbreviations: left frontal, left temporal, left occipital, right frontal, right temporal, right occipital.

perimental animals does not affect the validity of later electroencephalographic comparisons.

Three separate techniques for the production of acute increased pressure were employed:

(1) *The application of hydrostatic pressure to the cisterna magna.* A catheter was placed in the fourth ventricle and connected to a Y-shaped tube, one arm of which ran to a water manometer, the other to a reservoir of saline. By adjusting the height of the reservoir of saline the pressure applied to the brain could be controlled, and the pressure read in the manometer. At the end of the experiment methylene blue was added to the reservoir fluid in order to be certain that the system was patent. Since at autopsy the dye was found staining the surface of the fourth ventricle the technique was considered satisfactory.

(2) *Reducing the absorption of cerebrospinal fluid by lowering the osmotic pressure*

of the blood. Weed and Hughson have shown that the intravenous injection of distilled water produces a temporary increase in intracranial pressure, associated with brain edema (7, 8, 9). In order to read the intracranial pressure in terms of cm. of water, a catheter was placed in the cisterna magna and connected to a water manometer. Two intravenous injections of fifteen cc. of distilled water were given 15 minutes apart. After the second injection, records were taken at 15 minute intervals and the intracranial pressure noted.

(3) *Sudden occlusion of some of the veins draining the head.* Preliminary experiments showed that in order to increase the intracranial pressure, even temporarily, by venous ligation, it was necessary to occlude both internal and external jugular veins. Therefore, in this experiment, both internal jugulars were tied, and a loose ligature, laid under each external jugular, was brought through the skin, and tied very loosely. By turning a matchstick placed in the loose ligature a tourniquet effect produced occlusion of these veins and an acute increase in intracranial pressure.

Three cats were used. The electroencephalographic records were made on anaesthetized animals. The position and connections of the electrodes and the method of securing them to the scalp were similar to the technique described earlier.

Results

Hydrostatic pressure. The records in Fig. X show the effect of increased intracranial pressure produced by hydrostatic force. The pressure was raised from the normal 4–5 cm. of water to 50 cm. of water and maintained at the latter level for 20 minutes. It was necessary to reduce the pressure at the end of this time because of the danger of respiratory failure from medullary compression.

The electroencephalographic records show a pattern of low amplitude and high frequency, which quickly reverted to normal when the pressure was reduced. Large slow waves were never present.

These results confirm a similar experiment made on dogs (10). Increased intracranial pressure produced by hydrostatic force caused no change in the animals' electroencephalograms.

Intravenous distilled water. The records in Fig. XI show the effect of the development of 16 cm. of water pressure, during a period of two and one half hours. There is little or no change in the character of the waves. One may conclude that in cats, large slow waves are not associated with brain edema and an acute increase in intracranial pressure to 16 cm. of water.

Venous hypertension. In an attempt to produce maximum increased intracranial pressure by venous ligation, brain anoxemia is

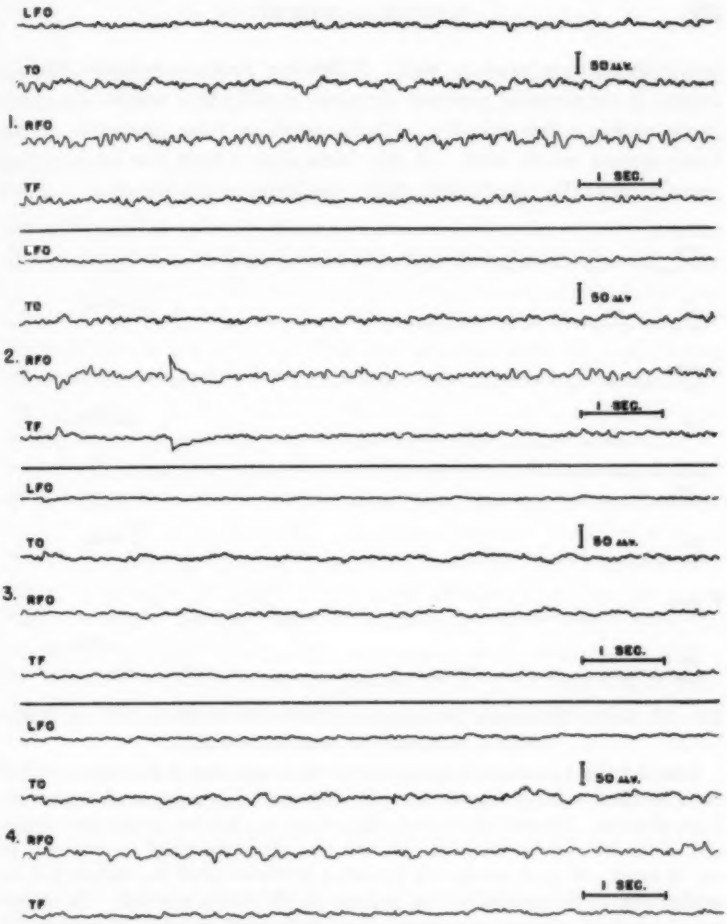

FIG. X. ACUTE INCREASED INTRACRANIAL PRESSURE PRODUCED IN A CAT BY HYDROSTATIC PRESSURE APPLIED THROUGH A CATHETER PLACED IN THE FOURTH VENTRICLE

Record 1 is a preliminary run when the pressure was set at zero. Record 2 was taken fifteen minutes after the hydrostatic pressure had been raised to 50 cm. of water. Note the decrease in amplitude and frequency in all the leads in comparison with the first sample. Record 3 was taken five minutes later under the same conditions as sample two. All activity is greatly reduced. Record 4 was taken a few minutes after the pressure had been reduced to normal.

These records show that acute increased intracranial pressure may exist without the occurrence of large slow waves in the electroencephalogram.

unavoidably produced as well. Following venous occlusion the increase in intracranial pressure develops rapidly and reaches its maximum within a few minutes. Coma resulting from anoxemia of the brain occurs much later. It has been shown that the brain waves remain virtually unaffected until considerable anoxemia is present

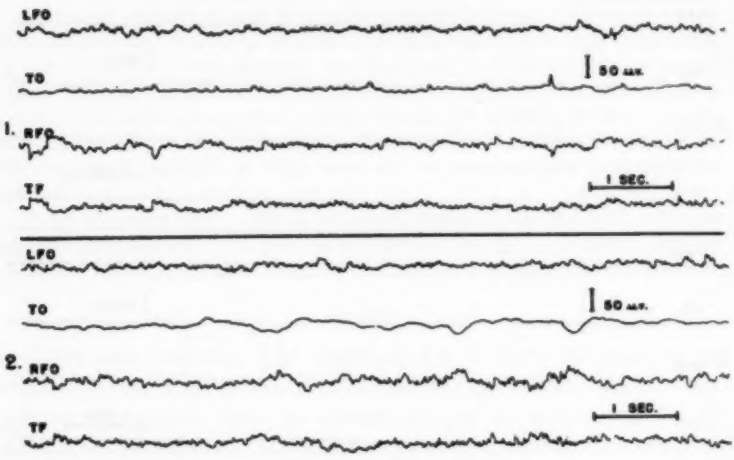

FIG. XI. ACUTE INCREASED INTRACRANIAL PRESSURE PRODUCED BY THE INTRAVENOUS INJECTION OF DISTILLED WATER

Record 1 was taken fifteen minutes after the completion of the injection of 30 cc. of distilled water intravenously. The intracranial pressure at this time was 5 cm. of water. Record 2 is a record taken two and a half hours after the completion of the intravenous injection. At this time the intracranial pressure was 16 cm. of water. In both records the potentials in the occipital channel are low, an artifact due to the presence of the catheter in the fourth ventricle. In neither record are large slow waves present. This indicates that increased intracranial pressure and edema may exist without the occurrence of large slow waves.

(11). If large slow waves were caused by this form of acutely increased intracranial pressure, one would expect to find them in the record shortly after occlusion of the veins. They might later be displaced by the flat pattern of anoxemic coma. In this experiment the venous occlusion was maintained until coma was reached, and this was repeated on two occasions. In the intervals the cat had time to

develop collateral circulation for better brain oxygenation at raised venous pressure. That this occurred was shown in the subsequent prolongation of the time interval between occlusion of the venous drainage and the development of coma.

In the first portion of the experiment following occlusion of the external jugular veins by means of a tourniquet, the pattern became flat as in coma. At this time a cisternal puncture was made. One cc. of cerebrospinal fluid came out under pressure and an additional 2 cc. were withdrawn. The brain waves approached normal but soon returned to the flat pattern. The cat appeared quite ill, and the record was discontinued. The right external jugular was permanently ligated. Only the left external jugular remained patent. At no time during the taking of the record were large slow waves present.

Four days later the experimental procedure was repeated. Under ether anaesthesia the intracranial pressure was measured and found to be 4 cm. of water (normal). A second base-line record was taken and the left external jugular again occluded. The record showed very flat waves on the left side. Fifteen minutes later the run was more nearly normal. During the next half hour the electroencephalogram showed a rhythmic wave of 20 per second which alternated with a flat record, indicating coma (Fig. XII, record 2). When the venous drainage was restored, the process was reversed; the flat waves giving way to the rhythmic 20 per second and finally to an almost normal record. Similar results are reported by Sugar and Gerard (11). Leads from the left and occipital areas (Fig. XII) were more nearly normal than those from the right, probably due to the fact that the right external jugular was still ligated.

A third and final run was made on the same cat eight days later. The cat during this time had both internal jugulars and the right external jugular occluded. Following a preliminary run the remaining vein was again occluded. The waves remained of low voltage. After the completion of the record, while the jugular veins were still occluded, the intracranial pressure was measured and found to be 10 cm. of water. At autopsy the brain was edematous and the veins distended.

In the experiment on this cat, complete occlusion of the jugular system was repeated three times within fourteen days. On each oc-

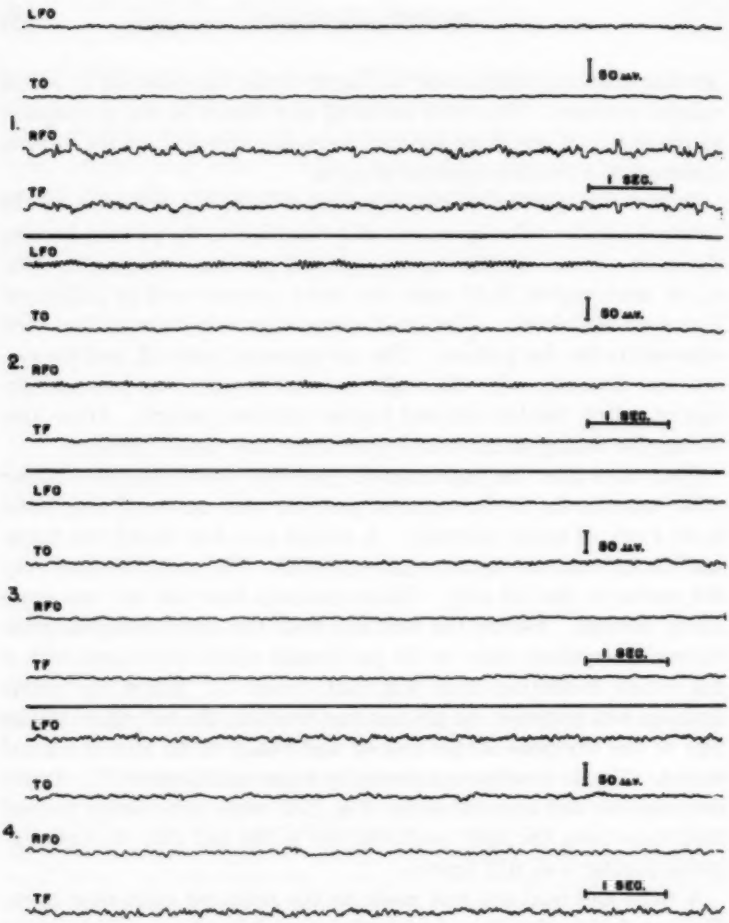

FIG. XII. CAT P. ACUTE INCREASED INTRACRANIAL PRESSURE RESULTING FROM THE OCCLUSION OF THE VENOUS DRAINAGE FROM THE HEAD

Record 1 was taken immediately after the occlusion of the remaining left external jugular vein. Note that the waves from the left hemisphere are extremely reduced. Since this change is unilateral it must be caused by anoxemia. This may be the explanation of its presence in moribund animals. Record 2, taken twenty minutes later, illustrates the regular 20 per second waves described in the text. Record 3, taken forty minutes after the occlusion, shows complete loss of waves. Since the animal was *in extremis* the venous drainage was restored. Record 4 shows almost normal brain waves present an hour later. The brain waves passed through a stage, similar to that seen in the second record, before returning to normal.

casion the time interval between occlusion and coma grew longer probably from the development of compensatory venous drainage. The likelihood of recording large slow waves, if they were to result from this form of increased intracranial pressure, consequently became greater. However, large slow waves never appeared in any of these records.

The results from these three techniques of raising the intracranial pressure suggest that increased intracranial pressure alone, causes brain waves of high frequency and low amplitude. It also appears that anoxemia causes first, a regular 20 per second rhythm in the brain waves, and later, a flat pattern of coma. Large slow waves were never present in the electroencephalograms of any of the three types of generalized increased intracranial pressure.

One may conclude that acute increased intracranial pressure may exist in cats without the occurrence of large slow waves in the electroencephalogram.

CHRONIC INCREASED INTRACRANIAL PRESSURE

Since hydrocephalus is a form of chronically increased intracranial pressure, it should be compared with an equally chronic form of generalized increased intracranial pressure. The present section makes this comparison and deals with the importance of the duration of the increased pressure.

Methods

The only satisfactory method for creating a chronic increase in cerebrospinal fluid pressure which could be satisfactorily employed was the ligation of a portion of the total venous drainage of the head. Therefore, the technique of venous ligation used earlier to produce an acute generalized increase in intracranial pressure was again used here. It was found necessary to ligate both posterior vertebral column sinuses, both internal jugular veins when they were of any size, and often one external jugular vein. The pressure fell slowly as the remaining venous channels grew larger, and new venous channels developed. This required a second and occasionally a third operation. With this technique the pressure was raised from the normal 4–5 cm. of water to 8–10 cm. as measured by cisternal puncture. This pressure was maintained for from four to five weeks. During the recording of the electroencephalograms the cats were lightly anaesthetized with Nembutal. The intracranial pressure was measured, whenever possible, at the end of a recording by means of cisternal puncture.

Results

Of the four cats, on which the procedure outlined above was performed, only one is illustrated (Fig. XIII). The records of the first cat covered 40 days. The intracranial pressure during this period varied from 8–10 cm. and averaged 9 cm. of water. This represents an increase of 4–6 cm. above the average normal. In a second cat, a total of 4 recordings were made at approximately weekly intervals over a period of 35 days. The intracranial pressure, measured after the last two records, was 7 and 8 cm., respectively. This represents an increase of 3–4 cm. above the average normal. In the third cat (Fig. XIII) five records were taken. The first record was taken prior to operation. The pressure at this time was 4–5 cm. of water. The next four records were taken at approximately weekly intervals over a period of 35 days. The pressure varied between 8 and 11 cm., averaging 9.5 cm. of water. In the fourth cat, four records were taken at approximately weekly intervals over a period of 30 days. The intracranial pressure varied from 7.5 to 12 cm. and averaged 9.0 cm. This represents an increase of from 2.0 to 7.5 cm. above the normal. If brain edema were the cause of large slow waves, one would expect them to be present in these records. All the records showed uniformly that in spite of increased pressure of from two to three times the normal, and its maintenance for a month, large slow waves did not appear.

This experiment illustrates a condition of chronic increased intracranial pressure not associated with large slow waves.

DISCUSSION

Two fundamentally different forms of increased intracranial pressure have been distinguished. In one form, active hydrocephalus, large slow waves were present; in the other, acute pressure up to 50 cm. of water and chronic pressures of 10 cm. of water, were produced without the occurrence of large slow waves. This distinction probably explains the lack of correlation of large slow waves with the height or duration of pressure. Earlier reports failed to distinguish between increased intraventricular pressure with hydrocephalus, and generalized increased pressure without hydrocephalus. Without this distinction, no correlation would appear to be possible.

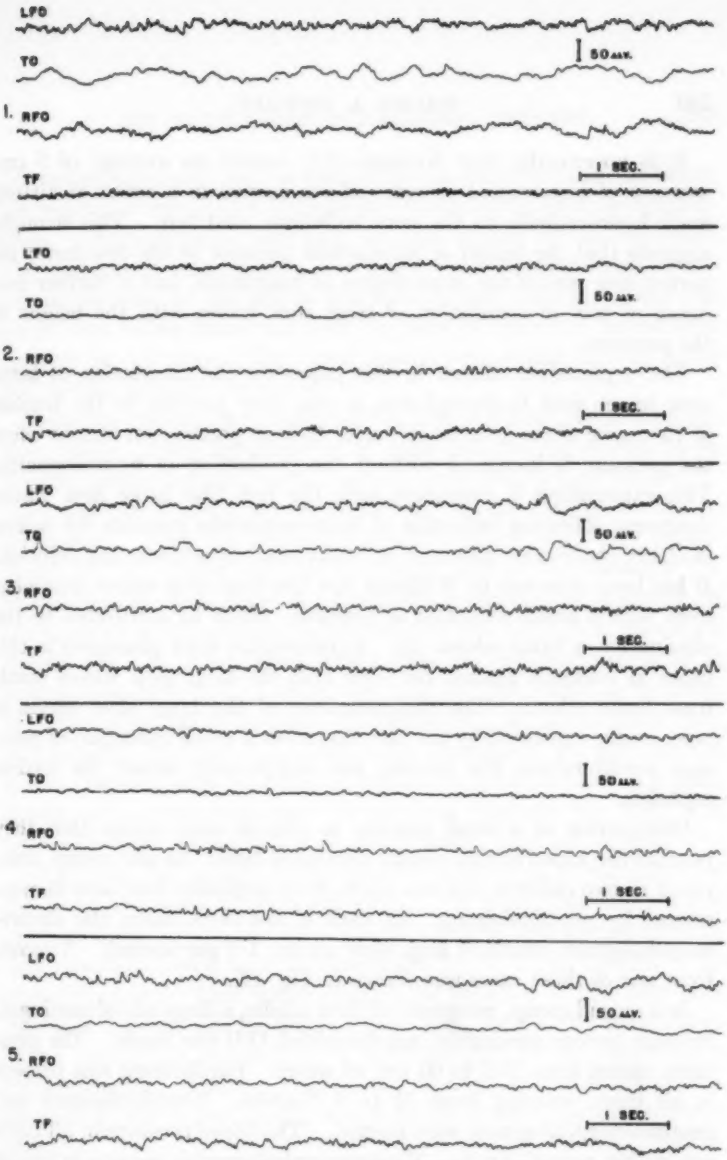

FIG. XIII. CHRONIC INCREASED INTRACRANIAL PRESSURE FROM VENOUS LIGATION

Record 1 is a normal electroencephalogram made before the posterior vertebral column sinuses were ligated. The intracranial pressure was 4–5 cm. of water. Records 2 and 3 were taken one and two weeks later, respectively. The intracranial pressure at this time was 11 cm. of water. Record 4 was made on the twenty-second day when the pressure was 8 cm. of water. On the twenty-eighth day both internal jugular veins and the left external jugular vein were ligated. Record 5 was taken five days later (thirty-two days after first operation) with the pressure at 10 cm. In spite of the chronic increased intracranial pressure which lasted thirty-two days, large slow waves were never present in the records.

It is noteworthy that Nanagas (12) reports an average of 5 cm. increase of intracranial pressure above normal in a series of kittens made hydrocephalic by the same technique used here. This strongly suggests that the height of intracranial pressure in the two forms reported here was of the same degree of magnitude, and is further evidence of lack of correlation of large slow waves with the height of the pressure.

The explanation offered in this paper for the correlation of large slow waves with hydrocephalus, is that they are due to the tension of the nerve fibres, present in active hydrocephalus, but absent when the pressure is increased without the production of hydrocephalus. This explanation is consistent with the fact that large slow waves disappear following reduction of intraventricular pressure by means of either ventricular puncture or intravenous hypertonic salt solution. It has been observed by Williams that the large slow waves disappear even with a small reduction of pressure, which he attributed to the elimination of brain edema (3). Experimental work presented in this paper is evidence against the view that the large slow waves result from brain edema. The disappearance of the large slow waves is more easily explained by the fact that even a small reduction of pressure would relieve the tension, and temporarily arrest the hydrocephalus.

Observation of a small number of clinical cases shows that they parallel the experimental results presented here. In one group, composed of two children and one adult, hydrocephalus had been demonstrated by ventriculogram. In each of the three cases, the electroencephalogram exhibited large slow waves, 1–5 per second. A record from one of these cases was shown in Fig. IX.

In a second group, composed of three adults, a diagnosis of meningeal hydrops (serous meningitis, arachnoiditis) (13) was made. The pressures varied from 29.5 to 60 cm. of water. Papilledema was present in all cases, varying from 2½ to 4 diopters. Ventriculograms and pneumoencephalograms were normal. The blood pressure in all cases was within normal limits. Electroencephalographic records in two of the cases were considered to be normal, with low amplitude. In the third case there were mild abnormalities, with fast activity. No large slow waves were present in any of the records.

Since in cases of compensated hydrocephalus (14) there is no actively

developing deformity and no tension on the nerve fibres of the brain, one would not expect to find large slow waves in the electroencephalogram. The writer has seen only one clinical case of this condition. The patient, an adult moron, had a history of headache and an abnormal increase in the size of the head during infancy. When the record was taken there had been no signs of increased intracranial pressure for 20 years. The brain waves were within normal limits and devoid of large slow waves.

There has been much speculation concerning the nature of the large slow waves. They are recorded in such pathological conditions as localized adhesive arachnoiditis, brain abscess, and cortical tumors (2). They have been described by Walter as "in the nature of inaction potentials." They are normally present in deep sleep (15), anaesthesia (11, 16), coma, and other types of loss of consciousness (2). In children at birth, there is incomplete myelinization of numerous tracts which are, therefore, non-functional, and large slow waves are present (2). In short, these waves seem to be present in cases of cortical inactivity. This inactivity may be functional, as in sleep, or pathological, as in localized arachnoiditis. In tumor cases (17), it is possible that the expanding lesion compresses the brain around it and causes a local increase in tension comparable to that present during active hydrocephalus. In the first instance, the large slow waves are local, while, in the second instance, they are general.

At present any answer to the general question as to the nature of the large slow wave must be conjecture. It is possible that cortical cells when inactive or partially damaged have a slow resting rhythm. The condition would then be comparable to the alpha rhythm of the occipital cortex. This would explain the presence of the large slow waves in persons asleep, under anaesthesia, or with hydrocephalus. When function is restored as on awakening or by the reduction of intraventricular pressure, this resting rhythm is broken up and regrouped in the faster smaller waves of the normal electroencephalogram.

SUMMARY

Abnormal large slow waves are present in the electroencephalogram of kittens developing hydrocephalus. Generalized acute or chronic increased intracranial pressure does not produce large slow waves.

Similar associations were found in the few clinical cases studied. An explanation is offered of the relation between the large slow waves and hydrocephalus. Based upon the evidence presented, it is believed that the large slow waves result from the tension of the white fibres of the brain, during active hydrocephalus. Their absence in other forms of increased intracranial pressure is accounted for by the absence of this tension.

In the concluding discussion the question is raised concerning the nature of the large slow wave and it is suggested that it represents a resting rhythm of the anterior portion of the brain.

REFERENCES

(1) BERGER, H.: Uber das Elektroenkephalogramm des Menschen. *Arch. f. Psycht.*, 1931, **94:** 16.

(2) WALTER, W. G.: Critical Review: The Technique and Application of Electroencephalogy. *J. Neurol. Psychiatry*, 1938, **1:** 359–385.

(3) WILLIAMS, D.: The Abnormal Cortical Potentials Associated with High Intracranial Pressure. *Brain*, 1939, **62:** 321–334.

(4) SCARFF, J. E., AND RAHM, W. E.: (*Personal communication.*)

(5) WEED, L. H.: Certain Anatomical and Physiological Aspects of the Meninges and Cerebrospinal Fluid. *Brain*, 1935, **58:** 383–397.

(6) WEED, L. H.: The Experimental Production of an Internal Hydrocephalus. *Contributions to Embryology No. 44.* Carnegie Institution of Washington, 1920, 425–446.

(7) WEED, L. H., AND MCKIBBEN, P. S.: Experimental Alteration of Brain Bulk. *Amer. Journ. of Physiol.*, 1919, **48:** 531–558.

(8) WEED, L. H., AND HUGHSON, W.: Systemic Effects of the Intravenous Injection of Solutions of Various Concentrations with Special References to the Cerebrospinal Fluid. *American Journal of Physiology*, 1921, **58:** 53–83.

(9) WEED, L. H., AND MCKIBBEN, P. S.: Pressure Changes in the Cerebro-Spinal Fluid Following Intravenous Injection of Solutions of Various Concentrations. *Amer. Journ. of Physiol.*, 1919, **48:** 512–530.

(10) HOAGLAND, H., HIMWICH, H., CAMPBELL, E., FAZEKAS, J., AND HADIDIAN, Z.: Effects of Hypoglycemia and Pentobarbital Sodium on Electrical Activity of Cerebral Cortex and Hypothalmus. *J. Neurophysiol.*, 1939, **2:** 276–288.

(11) SUGAR, O., AND GERARD, R. W.: Anoxia and Brain Potentials. *J. Neurophysiol.*, 1938, **1:** 558–572.

(12) NANAGAS, J. C.: Experimental Studies on Hydrocephalus. *Johns Hopkins Hospital Bulletin*, 1921, **32:** 1–28.

(13) FORD, F. R.: *Diseases of the Nervous System in Infancy, Childhood, and Adolescence.* Chapter 5, p. 429.

(14) FORD, F. R., AND MURPHY, E. L.: Increased Intracranial Pressure. *Bulletin of the Johns Hopkins Hospital*, 1939, **64**: 369–398.

(15) DAVIS, H., DAVIS, P. A., LOOMIS, A. L., HARVEY, E. N., AND HOBART, G.: Human Brain Potentials During the Onset of Sleep. *J. Neurophysiol.*, 1938, **1**: 24–38.

(16) RUBIN, M. A., AND FREEMAN, H.: Brain Potential Changes in Man During Cyclopropane Anesthesia. *J. Neurophysiol.*, 1940, **3**: 33–42.

(17) WALTER, W. G.: The Electroencephalogram in Cases of Cerebral Tumor. *Proc. R. Soc. Med.*, 1937, **30**: 579–598.

FAILURE OF PYRIDOXINE (VITAMIN B_6) TO MODIFY THE PARKINSONIAN SYNDROME*

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Following the isolation of crystalline vitamin B_6 (the rat anti-acrodynia factor) and the subsequent synthesis of this compound, it was not long before reports of the clinical effects of this vitamin began to appear in the literature. The first communication was that of Spies and his associates (1), who observed striking improvement in certain subjective symptoms such as nervousness, insomnia, irritability, abdominal pain, weakness, and difficulty in walking, within 24 hours after the administration of 50 milligrams of vitamin B_6 to four patients who had been successfully treated for pellagra (with nicotinic acid) and beriberi (with thiamin) but who had remained on deficient diets. This was followed by claims from various clinics that vitamin B_6 was effective in alleviating the symptoms of such heterogeneous disorders as arsenic polyneuritis, cheilosis, pernicious anemia and the macrocytic anemia of pellagra, pseudo-hypertrophic muscular dystrophy, and finally the parkinsonian syndrome. It seemed incredible that the deficiency of a single vitamin should be responsible for such a diverse group of unrelated diseases, no two of which are likely to develop in the same individual. Obviously further trial of the vitamin under carefully controlled conditions was indicated. The purpose of this paper is to report our experience in the treatment of the parkinsonian syndrome with crystalline vitamin B_6 (pyridoxine).

From two clinics favorable reports have appeared on the use of vitamin B_6 in Parkinson's syndrome. In the two groups comprising a total of 26 patients so treated, improvement, in the sense of relieving

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rigidity and increasing strength, was noted in a considerable number of cases. Spies and his associates (2) observed beneficial effects in three patients suffering from post-encephalitic parkinsonism, and in a few arteriosclerotic cases with long-standing paralysis agitans. Jolliffe (3), on the other hand, in his group of 15 patients found no improvement in the post-encephalitic cases and little or no change in patients with arteriosclerotic paralysis agitans completely disabled for longer than three years, but definite improvement was observed in patients with the latter type of disease who had been helpless for less than one year. In certain instances an amazing increase in function was noted within an hour after the intravenous injection of 50 mg. of the vitamin. In planning our study the observations of Spies and Jolliffe were given careful consideration.

CASE MATERIAL AND METHODS

Patients with post-encephalitic parkinsonism or arteriosclerotic paralysis agitans were selected from the neurologic clinic or the medical wards of the Johns Hopkins Hospital. Several criteria were applied in making this selection: 1) the patients should manifest outspoken characteristics of the parkinsonian syndrome; namely, cog-wheel rigidity, tremor, and loss of associated movements; 2) the symptoms should be of relatively short duration; 3) patients should be subjected to a trial of pyridoxine therapy only if they volunteered for such treatment after having the purely experimental nature of the procedure explained to them. The latter precaution was taken with the object of eliminating the psychological boost that so frequently accompanies the institution of any new form of treatment in such patients. Seven patients (four with arteriosclerotic paralysis agitans, two with post-encephalitic parkinsonism, and one in whom the exact etiology was obscure) were chosen for a prolonged trial of intravenous therapy with vitamin B_6; five of the seven entered the hospital, the other two were treated in the out-patient clinic. A dietary history was taken in every case. A careful neurologic examination was performed on each patient prior to starting treatment and repeated frequently during and after the period of vitamin therapy. Pyridoxine (vitamin B_6) hydrochloride (Merck) was dissolved in sterile normal saline or distilled water and administered intravenously in all cases. Five

patients were given 50 mg. a day for two weeks, one patient received 50 mg. a day for one week, and one received 100 mg. a day for one week. Three additional patients were given a single intravenous injection of 100 mg. of vitamin B_6 hydrochloride and were observed for several hours following the injection for possible immediate effects upon the parkinsonian symptoms.

In 6 of the 7 patients treated with repeated injections of pyridoxine the urinary excretion test of Scudi and his associates (4) was carried out at the time of the first injection and repeated at intervals during the course of therapy. Spies (5) has reported that normal individuals excrete at least 8 per cent of the injected dose in the first hour, whereas patients showing evidence of deficiency of vitamin B_6 will excrete little or none of the test dose.

CASE ABSTRACTS

Case #1. F. T., male, white, aged 25 years. In January, 1937 the patient developed "sleeping sickness" and slept 9½ months. When he regained consciousness, his movements were slow, stiff, and awkward. He had to learn to walk all over again. On three subsequent occasions he slept for periods varying from 9 days in April, 1938, to 53 days in October, 1938. Early in 1940 he was asleep for 2–3 weeks. When admitted to the hospital in the fall of 1940 he complained of drowsiness and sharp pains over his forehead. He was obliged to take a nap every 2–3 hours during the day, but experienced some difficulty in sleeping at night. His diet had been good.

On physical examination the temperature, pulse, and respiration were normal. Blood pressure was 110/65. Nutrition was good. Activity was slow; his voice was low-pitched and his speech was difficult to understand. The face was mask-like; there was a moderate stoop. Marked rigidity of the muscles of the extremities was present. There was a tremor of the eyelids. Although the deep tendon reflexes were not exaggerated and the abdominal reflexes were active, there was a bilaterally positive Babinski sign. Blood count and urine were normal; the Wasserman test was negative.

Impression: Chronic encephalitis lethargica with parkinsonian syndrome.

Treatment: The patient was first given hyoscine 1.2 mgm. and benzedrine sulfate 10 mgm. daily. He became brighter and more cheerful; his reactions were quicker. The hyoscine and benzedrine were continued during a subsequent 2-week period of daily intravenous injection of 50 mgm. of crystalline vitamin B_6. There was no further improvement in the patient's condition under the vitamin therapy. The patient was eventually given large doses of atropine in addition to the hyoscine and benzedrine with beneficial effects.

Case #2. C. S., female, white, aged 33. In March, 1937 this multiparous housewife first noticed shaking of her left arm during the sixth month of her fourth pregnancy. No definite history of preceding encephalitis was obtained. Shortly after the tremor had developed in her arm, the left leg began to shake and she experienced difficulty in speaking. Hypersalivation became troublesome. Over the ensuing years the tremor and stiffness of the left arm and leg increased and the right arm and leg became similarly involved to a lesser extent. As far as could be ascertained, her diet had always been adequate. Prior to the trial of vitamin B_6 therapy she had received hyoscine hydrobromide (as much as 9 mg. a day) without much improvement.

Physical examination in October, 1940 revealed a slender woman who was seriously handicapped by the coarse tremors of all four extremities, more pronounced on the left. There was striking cog-wheel rigidity, and loss of associated movements when she walked. The dysarthria, resulting from the coarse tremor of the tongue and laryngeal muscles, was so marked that her speech was almost unintelligible. Blood pressure was 130/85, blood count and urine normal, Wassermann reaction negative.

Impression: Post-encephalitic parkinsonism.

Treatment: On November 2, 1940, daily intravenous injections of 50 mg. vitamin B_6 hydrochloride were started and continued for two weeks. The dose of hyoscine hydrobromide (9 mg. a day) which she had been receiving prior to vitamin therapy was continued throughout. She showed absolutely no improvement during the period of treatment with vitamin B_6.

Case #3. J. L., male, white, aged 69 years. In 1937 he noted that he was "slowing down" and his limbs were growing stiff. Tremor of the hands developed. Talking became an effort. He experienced difficulty in performing finer movements such as dressing himself. There was no history of encephalitis. His appetite remained good and his diet was excellent. Stramonium and benzedrine had been administered with little effect; he was somewhat better on hyoscine. In September, 1940 he was admitted to the hospital for a trial of vitamin B_6 therapy. In the previous three weeks he had taken no form of medication.

Physical Examination: Development and nutrition were good. There was well-marked arteriosclerosis. Blood pressure was 140/90. The patient lay flat in bed and made few spontaneous movements. He was unable to turn over or sit up without assistance. He could turn his head slightly from side to side but was unable to bend it forward. The face was expressionless. Speech was slow and thick. There was a slow pill-rolling tremor of both thumbs and striking generalized rigidity with cog-wheel phenomenon at the wrists and elbows. Reflexes were brisk and equal on the two sides. Blood count and urine were normal, the Wassermann negative.

Impression: Paralysis agitans on an arteriosclerotic basis.

Treatment: The patient was given a daily intravenous injection of 50 mgm.

vitamin B_6 for one week. He was just as helpless at the end of the week as before treatment. Therefore the injections were discontinued and "Rabellon"[1] was administered by mouth in gradually increasing doses until he was receiving 1.5 mgm. a day. The rigidity and tremor both decreased and the patient became considerably more active. He was able to turn over in bed, get up unassisted, and dress himself. He could walk fairly well without assistance.

Case #4. H. B., female, colored, aged 68 years. In 1937 the patient first noticed a coarse tremor of her hands. There was no history of encephalitis. Her movements gradually became slower and more awkward. Early in 1940 she became stooped and developed a propulsive gait so that she could not avoid running after she had walked a few steps. Although no accurate dietary history was obtainable owing to the patient's mental status, it was evident that her appetite had never failed her. She was admitted to the hospital in November, 1940.

Physical Examination: The patient was a short well-nourished elderly colored woman who stood with a marked stoop. Although her movements were all slow, the gait was characteristically propulsive with loss of associated movements of the arms. There was a coarse rhythmic tremor of the hands and pronounced cog-wheel rigidity in both arms and legs. The blood-pressure was 180/80. The arteries showed advanced sclerosis. The teeth were all carious. The blood count and urine were normal; the blood Wassermann was positive, but the spinal fluid Wassermann was negative. There was gastric achlorhydria, even after stimulation with histamine.

Impression: Paralysis agitans on an arteriosclerotic basis; dental caries; achlorhydria gastrica.

Treatment: The patient was given an intravenous injection of 50 mgm. of vitamin B_6 daily for 15 days. Although she stated that she felt a little better during this period, no objective evidence of improvement could be made out. After the trial of vitamin therapy she was given "Rabellon" by mouth in doses up to 2 mgm. a day with striking subjective and objective improvement.

Case #5. C. C., female, white, aged 58 years. In 1936 the patient's left arm and leg began to shake. There was no history to suggest a preceding encephalitis. In 1938, the blood Wassermann test was found to be positive and antisyphilitic treatment was administered over the next two years. The spinal fluid examination was normal before and after treatment. The tremor of the left arm and leg persisted and in the summer of 1940 the extremities on the right side began to shake as well. Her diet had been good. She was admitted to the hospital in November, 1940.

On *physical examination* the following abnormalities were noted: blood pressure

[1] *Rabellon* (Sharp and Dohme) is a combination of three belladonna alkaloids: hyoscyamine, atropine, and scopolamine.

185/95, obesity, arteriosclerosis, slight cardiac enlargement, hypertrophic arthritis of the spine. The neurologic examination revealed an expressionless face, coarse parkinsonian tremor of the arms and legs (more pronounced on the left side), cog-wheel rigidity in all four extremities, and hyperactive deep reflexes. Blood count and urine were normal.

Impression: Paralysis agitans probably on an arteriosclerotic basis: hypertension; latent syphilis.

Treatment: The patient was given an intravenous injection of 50 mgm. of crystalline vitamin B_6 daily for two weeks. There was no subjective or objective improvement during this period. She was discharged from the hospital on "Rabellon" 2 mgm. a day, but experienced no definite benefit from this form of treatment.

Case #6. N. B., male, white, aged 60 years. The patient gave a history of having "grippe" for three days in April, 1939. One month later his right arm began to shake and shortly thereafter the right side of his face felt stiff and a tremor developed in the right leg. The stiffness gradually increased and he noted a tendency to fall forward when he walked. His diet was well-balanced. The patient was admitted to the hospital in December, 1940 for a trial of vitamin B_6 therapy.

Physical Examination: An undernourished white man of 60 with well-marked arteriosclerosis; blood pressure was 125/75. The right side of the face was expressionless. There was a coarse parkinsonian tremor of the right arm and leg with pronounced cog-wheel rigidity in both of these limbs. The right arm did not swing when he walked. There was practically no involvement of the left side of the body. The deep reflexes were brisk, more active on the right than the left. The blood count, sedimentation rate, and urine were normal. The blood Wassermann and spinal fluid were negative.

Impression: Paralysis agitans, right-sided, on an arteriosclerotic basis seemed most likely, although a post-encephalitic parkinsonism was considered possible in view of the history.

Treatment: Since there had been no improvement in the earlier cases treated with 50 mgm. vitamin B_6 daily, it was decided to give this patient 100 mgm. of the vitamin intravenously every day. This program was followed for one week during which time the patient showed absolutely no improvement, subjective or objective. The patient developed edema of the ankles and his urine showed a few red blood cells and casts. Although there was no proof that the vitamin B_6 was responsible for these changes, the injections were discontinued. The edema disappeared and the urine cleared up soon after. The patient was then given hyoscine 4 mgm. a day with rapid and striking improvement.

Case #7. A. G., male, white, aged 69 years. In 1937 the patient noticed that he was not swinging his left arm when he walked. A year later he developed some

pain in his left arm and shoulder; the left arm and leg began to shake. The symptoms were always worse when he was nervous or tired; they interfered with his sleep. There was no history of encephalitis. The diet had been good.

Physical Examination: The patient was a slender elderly white man who walked with a considerable stoop. The left arm did not swing and the left leg dragged a little. A slow coarse tremor of the left hand was present. There was striking cog-wheel rigidity of the left arm and leg. The right hand showed a very slight tremor, but there was no appreciable rigidity of the right arm or leg. The deep reflexes were brisk, more active on the left. Blood pressure was 140/80. Blood count and urine were normal, the Wassermann negative.

Impression: Paralysis agitans, chiefly left-sided, on an arteriosclerotic basis.

Treatment: Period 1: 1st to 15th day: Daily intravenous injection of 50 mgm. vitamin B_6 dissolved in 5 cc. physiologic saline. There was striking subjective improvement (patient less nervous, appetite increased, sleep better) following the initial injection. This persisted during the entire period. At no time was there objective evidence of improvement in the rigidity, tremor, or gait.

Period 2: 16th to 36th day: Daily intravenous injection of 5 cc. physiologic saline. Patient continued to feel better but there was no objective change.

Period 3: 37th to 57th day: No therapy. Two days after the injections were stopped, the patient felt that he was losing ground: subjectively shaking more, stiffer; sleep restless; appetite poor. There was no objective change in symptoms or signs during this period.

Period 4: 58th to 71th day: Daily intravenous injection of 5 cc. physiologic saline (no vitamin B_6). Once more the patient felt very much better just as he did during the original course of vitamin B_6 therapy. It was quite apparent that the daily intravenous injections exerted a tremendous psychologic effect which was independent of the vitamin B_6 content.

At no time during the 10-week period of observation was there any objective change in the neurologic status. All intravenous treatment was finally discontinued and the patient was given 0.3 mgm. hyoscine hydrobromide three times a day. Under this therapy there was for the first time marked reduction in the rigidity and tremor along with pronounced subjective improvement.

RESULTS

Seven patients with outspoken manifestations of the parkinsonian syndrome have been treated with intravenous injections of vitamin B_6 over periods of one to two weeks. The results are summarized in Table I. In no case was the slightest evidence of objective improvement obtained, and in only one case (case # 7) was there an appreciable degree of subjective improvement. The latter patient was subsequently retested with intravenous injections of normal saline containing no vitamin B_6. The subjective response to the saline injections

was in every way comparable to that observed during the period of vitamin B_6 therapy, suggesting that the mere stimulus of an intravenous injection exerted an unusually powerful effect upon the psyche of this individual.

TABLE I

CASE NO.	RACE	SEX	AGE	DIAGNOSIS	DURATION OF SYMPTOMS	DURATION OF B_6 THERAPY	TOTAL AMOUNT B_6	URINARY EXCRETION OF PYRIDOXINE (PER CENT OF TEST DOSE EXCRETED IN ONE HOUR)			RESULT
								1st day	7th day	14th day	
					years	*weeks*	*mg.*	*per cent*	*per cent*	*per cent*	
1	W	M	25	Post-encephalitic parkinsonism	3½	2	700*	10.4	20.0	19.0	No improvement
2	W	F	33	Post-encephalitic parkinsonism	3½	2	700*		21.8	19.6	No improvement
3	W	M	69	Arteriosclerotic paralysis agitans	3	1	350*	8.6	11.9		No improvement
4	C	F	68	Arteriosclerotic paralysis agitans	3	2	700*	17.8	15.6	12.3	Slight subjective improvement; no objective change
5	W	F	58	Arteriosclerotic paralysis agitans	4	2	700*	10.8	10.9	8.1	No improvement
6	W	M	60	Arteriosclerotic paralysis agitans	1½	1	700†	7.9	15.2		No improvement
7	W	M	69	Arteriosclerotic paralysis agitans	3	2	700*	13.9	23.2	22.3	Marked subjective improvement, but no objective change. Subsequent striking subjective improvement on intravenous salt solution alone

* 50 mg. per day.
† 100 mg. per day.

In four of the patients quantitative measurements of the muscular strength in the arms were made by Dr. Orthello Langworthy. No increase in strength occurred during the period of vitamin B_6 therapy. In six of the seven patients the urinary excretion of pyridoxine during the first hour following the initial injection was measured according to the method of Scudi and his associates (4). We are not prepared to state whether or not this test is in any way a measure of vitamin B_6 deficiency, but merely wish to point out that in all six patients the amount of the vitamin excreted was well within what is considered the normal range by both Scudi (4) and Spies (5). In this respect our results differ from those of Flexner and Chassin (6) who found the urinary excretion of pyridoxine following the intravenous injection of a 50 mgm. test dose to be reduced below normal in 6 or 7 cases of post-encephalitic parkinsonism. It is interesting to note that in our cases at the end of the treatment periods only in 3 of the 6 cases was there an appreciable increase in the percentage of the 50 mg. dose excreted over the initial excretion level.

Three additional patients with post-encephalitic parkinsonism were each given a single intravenous injection of 100 mg. of vitamin B_6 hydrochloride and were observed for several hours thereafter. In no case did the slightest improvement manifest itself. Thus the observation of Jolliffe that immediate alleviation of symptoms may follow such treatment could not be confirmed in a single instance among the ten cases that comprise our series.

DISCUSSION

In the cases which we have reported there was no reason to suspect from the dietary histories of the patients, their clinical appearance, or the urinary excretion test (if this be of any value) that they were suffering from deficiency of vitamin B_6 or of any of the other factors of the vitamin B complex which are so closely associated with one another in their natural food sources. In view of this it is not surprising that treatment with pyridoxine was of no benefit in any of the cases. Zeligs (7) has likewise failed to confirm the original favorable reports. He treated 10 cases of post-encephalitic parkinsonism and 5 patients with arteriosclerotic paralysis agitans by giving daily intravenous injections of 50 to 100 mg. of pyridoxine hydrochloride. He took care to avoid playing upon the suggestibility of his patients.

Flexner and Chassin (6) did not state whether treatment was beneficial in their cases. Sebrell (8) has informed us that he has treated 24 cases of severe long-standing parkinsonism with pyridoxine over periods of 2 weeks each, but in no case has objective improvement been observed. On the other hand, Baker (9) has very recently reported objective improvement in 6 of 15 patients with paralysis agitans treated with intravenous injections of vitamin B_6, and subjective improvement in 2 of 4 patients given pyridoxine orally. Neither the age of the patient nor the duration of symptoms seemed to bear any relationship to the effect of treatment. Most of the patients were treated for longer periods than those we have reported, and improvement was first noted after several weeks in certain cases. It is difficult to see how improvement which first became evident six weeks after completion of a course of pyridoxine in one patient could be attributed to the effects of the vitamin.

In spite of the conflicting reports in the literature it is our opinion that the preponderance of evidence fails to support the rationale of pyridoxine therapy in the parkinsonian syndrome.

CONCLUSION

Vitamin B_6 proved to be totally ineffective in the treatment of the parkinsonian syndrome in a selected group of ten patients.

REFERENCES

1. Spies, T. D., Bean, W. B., and Ashe, W. F.: A note on the use of vitamin B_6 in human nutrition. *J. A. M. A.*, **112:** 2414, 1939.
2. Spies, T. D., Hightower, D. P., and Hubbard, L. H.: Some recent advances in vitamin therapy. *J. A. M. A.*, **115:** 292, 1940.
3. Jolliffe, H.: Clinical aspects of vitamin deficiencies. *Minn. Med.*, **23:** 542, 1940.
4. Scudi, J. V., Unna, K., and Antopol, W.: A study of the urinary excretion of vitamin B_6 by a colorimetric method. *J. Biol., Chem.*, **135:** 371, 1940.
5. Spies, T. D., Ladisch, R. K., and Bean, W. B.: Vitamin B_6 (pyridoxin) deficiency in human beings. *J. A. M. A.*, **115:** 839, 1940.
6. Flexner, J., and Chassin, M. R.: Clinical studies on pyridoxine (vitamin B_6). *J. Clin. Investigation*, **20:** 313, 1941.
7. Zeligs, M. A.: Use of pyridoxine hydrochloride (vitamin B_6) in parkinsonism. *J. A. M. A.*, **116:** 2148, 1941.
8. Sebrell, W. H.: Personal communication to the authors.
9. Baker, A. B.: Treatment of paralysis agitans with vitamin B_6 (pyridoxine hydrochloride). *J. A. M. A.*, **116:** 2484, 1941.

ON A GRAPHIC SOLUTION OF THE DOSAGE-EFFECT CURVE[1]

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Several methods for the approximate evaluation of a dosage-effect curve have been proposed. The median effective dose, $E.D._{50}$, is generally accepted as the basic value to be obtained from the dosage-effect curve. This value can be interpolated graphically from the curve according to the procedure of Trevan (1), or Gaddum (2). It may also be obtained without representation of the curve by the procedure of Dragstedt (3), Behrens (4), or Karber (5). It is, however, impossible to assess the accuracy of the $E.D._{50}$ obtained by the above methods. With a restricted experimental design Karber's method does yield an estimate of the standard error.

If the percentage effects at the various doses are converted into relative deviates of a normal curve, or normal equivalent deviations in Gaddum's terminology (2), and plotted against dosage or some suitable function of it such as the logarithm, the dosage-effect curve will be linear. In Bliss's procedure (6) the percentages are converted into probits, i.e., normal equivalent deviations plus five. From the free-hand line drawn through these points, the parameters $E.D._{50}$ and the slope b can be read off directly. The validity of a bioassay based on a dosage-effect curve depends on 1) the parallelism between "standard" curve and "unknown" curve and 2) the sampling error of the ratio of "standard" to "unknown" potency. Therefore, it is essential to know the standard error of the slope and of the median effective dose. These standard errors have been obtained generally by mathe-

[1] This investigation has been aided by a grant from The John and Mary R. Markle Foundation.

matically fitting the dosage-effect curve. It seems desirable to review briefly the method of fitting:

In Gaddum's procedure each normal equivalent deviation is assigned a weight w, the inverse of the variance of the percentile, nz^2/pq, where n is the number of animals at that dose, p is the proportion surviving, q the proportion dying, and z is the ordinate of the unit normal curve corresponding to a proportion p. The line is then fitted by least squares. In Bliss's procedure the weights are determined by the probits at the various doses as read from a provisional line drawn through the points rather than by the observed probits. Furthermore, these probits on the provisional line are made the basis for certain continuity corrections to the observed probits as deduced from maximum likelihood considerations. The line is then fitted by least squares using these corrected probits and their weights. The equation of the fitted line is

$$Y = a + b(x - \bar{x}) \qquad \text{I}$$

where Y is the predicted probit and x the chosen function of the dosage, say log dosage. Here, $\bar{x}$ is the average log dose evaluated as

$$\bar{x} = S(wx)/S(w) \qquad \text{II}$$

where S indicates summation over all the points. From the least square solution

$$a = \bar{y} = S(wy)/S(w) \qquad \text{III}$$

$$b = S[wy(x - \bar{x})]/S[w(x - \bar{x})^2] \qquad \text{IV}$$

Significant departure of the points from the fitted line indicating that the best fit has not yet been attained or that heterogeneity exists may be tested by a Chi-square criterion utilizing the deviations of the points from the line. If the fitted line differs greatly from the provisional line or is otherwise unsatisfactory, a second line may be fitted, using the first as a basis for obtaining a new series of weights and corrected probits. Log E.D.$_{50}$ will then be that value of x for which $Y = 5$, and may be derived as

$$\text{Log E.D.}_{50} = \bar{x} + \frac{5 - \bar{y}}{b} \qquad \text{V}$$

Provided that the deviations of the points from the line do not indicate heterogeneity, the standard errors of log E.D.$_{50}$ and b may then be deduced.

$$\text{S.E.}_b^2 = 1/S[w(x - \bar{x})^2] \qquad \text{VI}$$

If the experiment was well planned so that the average log dose is not far from log E.D.$_{50}$, then

$$\text{S.E.}_{\log \text{E.D.}_{50}}^2 = \frac{(5 - \bar{y})^2}{b^4 S[w(x - \bar{x})^2]} + \frac{1}{b^2 S(w)} \qquad \text{VII}$$

Routine use of Gaddum's method or of Bliss's more elaborate method is laborious even with the aid of a calculator. It is the purpose of this paper to compare graphical estimates of the parameters log E.D.$_{50}$ and b with those obtained by the least square solution and to present certain approximations to the standard errors of these parameters. The comparison is based on 54 dosage-effect curves.

The experimental data from which these curves were derived were obtained with the use of several strains of mice. The number of mice used to determine any one curve varied from 27 to 120. The number of animals used at any particular dose varied from 3 to 12. The object of each of the experiments was to relate curative effect to dose of a drug, in the presence of an infection which was invariably fatal to untreated mice. The experimental procedure used in obtaining these data has been previously reported (7). Seven different sulfanilamide derivatives and two different bacterial infections are represented in this series. The experiments were carried out over a two-year period.

PROCEDURE

The data of each experiment were treated as follows. Doses were converted to logarithms and per cent survival to probits, by means of suitable tables (6). The values obtained were plotted on arithmetic coordinate paper and a free-hand straight line was drawn through the points, using a transparent straight edge to facilitate placing the line to best advantage. The logarithm of the E.D.$_{50}$ (the dose at which an ordinate would intersect the line at probit value 5) was then read from the graph.

The slope of the line can readily be estimated as

$$b = (y_2 - y_1)/(x_2 - x_1)$$

where (x_1, y_1) and (x_2, y_2) are any two points on the line. The reciprocal of the slope, s, is the standard deviation of the individual effective doses and represents the dosage range required to alter the response by one probit.

The approximations to the standard errors of log E.D.$_{50}$ and b were obtained as follows:

From formula VII it is evident that if y is near 5, the contribution of the first part of the formula is small compared with the second part. Thus S.E.^{2} log E.D.$_{50}$ is approximately $s^2/S(w)$ where s has already been estimated ($S = 1/b$). $S(w)$ is composed of terms like $n(z^2/pq)$. Reference to a table of the normal curve or of weights (6) shows that (z^2/pq) has a value of .6366 at 5 probits, decreasing to .4386 at 4 or 6 probits and rapidly decreasing thereafter. We shall not be far wrong if we take a value of .5 for all animals within s units of log E.D.$_{50}$, ignoring the weight of the other points. The resultant underestimation of $S(w)$ partly compensates for any discrepancy of $\bar{y}$ from 5. Thus an entirely satisfactory formula appears to be

$$\text{S.E.}_{\log \text{E.D.}_{50}} = s \Big/ \sqrt{\frac{N'}{2}} \qquad \text{VIII}$$

where N' is the number of animals within s units of log E.D.$_{50}$.

It is more difficult to obtain an adequate approximation to S.E.$_b$. Formula VI may be rewritten as

$$\text{S.E.}_b^2 = \frac{1}{S(w) \cdot \sigma_x^2}$$

where σ_x is the standard deviation of the distribution of the logarithms of the doses. This distribution if plotted would be represented by a series of ordinates proportional to the weights at the respective log doses. Well-planned experiments will have the logarithms of the doses fairly uniformly spaced with the average not far from log E.D.$_{50}$. If the range of doses is limited to those contributing appreciable weight, say from the highest consistently giving zero per cent effect to the lowest consistently giving 100 per cent effect, then the distribu-

tion will be roughly a semicircle. Considering this semicircle as a continuous distribution, the standard deviation is $L/4$, where L is the range. The discrete distribution of doses may be made essentially continuous by replacing each ordinate by a bar centered at the appropriate dose and of area equal to the ordinate. With k evenly spaced doses this procedure amounts to increasing the range between the highest and lowest dose by a distance equal to $100/k - 1$ per cent. Thus we may take

$$\sigma_s = \frac{Lk}{4(k-1)}$$

TABLE 1

Sulfapyridine Treatment of a Streptococcus Infection in Mice

DOSE	NUMBER OF MICE	PER CENT SURVIVAL	LOGARITHM OF DOSE	PROBIT OF PER CENT SURVIVAL
mgm.				
7.6	5	80	0.88	5.84
6.0	5	60	0.78	5.25
4.1	5	60	0.62	5.25
3.0	5	40	0.47	4.75
1.6	5	20	0.19	4.16
1.0	4	0	0.00	—

Using the same approximation to $S(w)$ as previously, namely, $N'/2$, we have

$$\text{S.E.}_b = \frac{4}{\sqrt{\frac{N'}{2}}\, L \frac{k}{k-1}} \qquad \text{IX}$$

The range L is taken as the difference between the largest of the low doses which gave zero per cent effect and the smallest of the high doses which gave 100 per cent effect.

However, for the following cases, if 1) zero per cent or 2) 100 per cent effect or 3) both are not observed in the experiment, the range is delimited respectively by 1) the lowest or 2) the highest dose or 3) both. If a zero or 100 per cent effect occurs within the limits of the

effective (from zero to 100 per cent) range, it is disregarded for purposes of establishing the range L, and the above criteria are used.[2]

The use of this procedure is demonstrated by the following example. The data of one experiment are given in table 1, the last two columns of which are plotted in fig. 1. The line is drawn through these points and ordinates are erected at log doses corresponding to probit levels of 4, 5 and 6. The logarithm of the E.D.$_{50}$ is read from the dosage scale as 0.570. Two points, (x_1, y_1) and (x_2, y_2), on the line are selected,

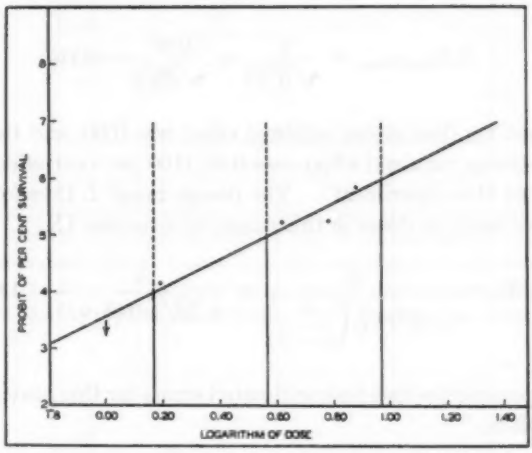

FIG. 1. Free-hand dosage-effect curve for sulfapyridine treatment of a streptococcus infection in mice. Ordinates have been erected at doses corresponding to probit levels of 4, 5 and 6.

e.g., $x_1 = \bar{1}.80$, $y_1 = 3.09$, and $x_2 = 1.20$, $y_2 = 6.58$. The slope of the line is now estimated as:

$$b = \frac{6.58 - 3.09}{1.20 - 1.80} = \frac{3.49}{1.40} = 2.49$$

[2] This case will be made clear by the following example in which the observed per cent effects at the various doses were: 0, *0*, 10, 0, 30, 60 and *80*. The zero per cent, lying between 10 and 30 per cent effects is disregarded, and L is taken as the difference between the highest log dose (at 80 per cent effect) and the larger of the two log doses causing zero per cent below the 10 per cent effect. The effects representing these limits have been italicized above.

The standard deviation of the individual responses is the reciprocal of the slope b:

$$s = \frac{1}{b} = \frac{1}{2.49} = .401$$

There are 5 doses falling between the ordinates to the curve corresponding to probit values 4 and 6. Since 5 animals were used at each of these doses, the value of N′ is 25. Then by equation VIII

$$\text{S.E.}_{\log \text{E.D.}_{50}} = \frac{s}{\sqrt{N'/2}} = \frac{0.40}{\sqrt{25/2}} = 0.116$$

The largest log dose giving minimal effect was 0.00, and the smallest log dose giving maximal effect was 0.88 (100 per cent effect was not observed in this experiment). The dosage range L therefore is 0.88. Since there were six doses in this range, by equation IX:

$$\text{S.E.}_b = \frac{4}{\sqrt{N'/2}\, L\left(\frac{k}{k-1}\right)} = \frac{4}{\sqrt{25/2}\cdot 0.88\cdot 6/5} = 1.07$$

The two parameters and their estimated errors for this provisional line are, therefore,

$$\text{Log E.D.}_{50} \pm \text{S.E.}_{\log \text{E.D.}_{50}} = 0.57 \pm 0.116$$

$$b \pm \text{S.E.}_b = 2.50 \pm 1.07$$

RESULTS

Estimated parameters and their errors were obtained from the data of 54 dosage-effect curves by the simple procedure illustrated above. The parameters and their errors were then calculated from these same data by the method of Bliss (6), using the same free-hand dosage-effect curve as the provisional curve. Thus the estimated values already obtained are free of bias with respect to foreknowledge of the later computed values. The fitting process was continued in each case

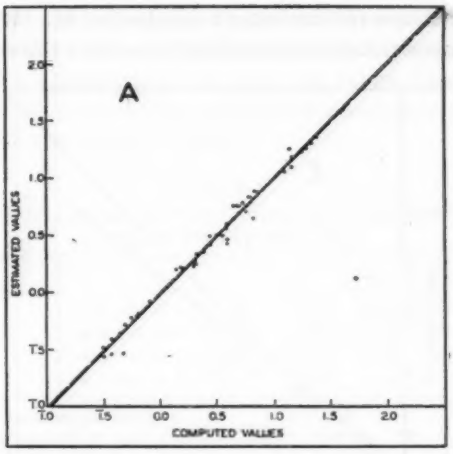

FIG. 2. Regression of estimated on computed values from 54 dosage-effect curves of: A, the logarithm of the median effective dose; B, the standard error of the logarithm of the median effective dose; C, the slope constant, b; D, the standard error of the slope constant. Each point represents the appropriate values from one dosage-effect curve. Broken line indicates true regression line with slope constant of 1.000.

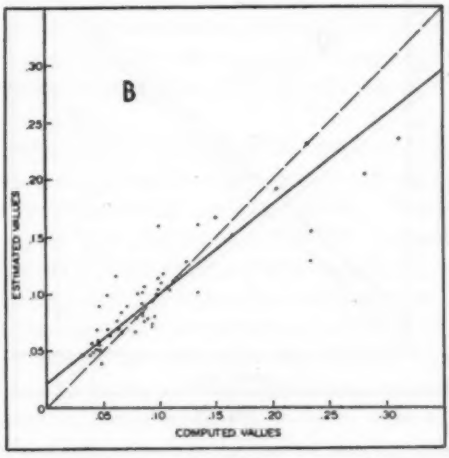

FIG. 2B

until the Chi-square test indicated a satisfactory fit. With few exceptions a single computation was sufficient to achieve this end. In fig. 2A

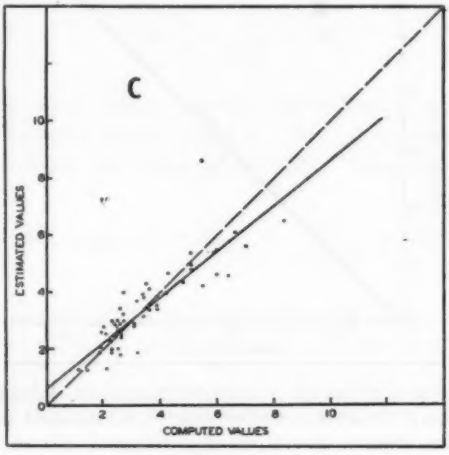

FIG. 2C

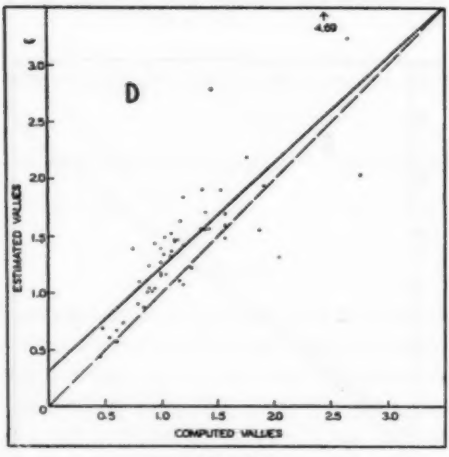

FIG. 2D

the estimated values of log E.D.$_{50}$ are plotted against their corresponding computed values. Estimated values of S.E.$_{\log \text{E. D.}_{50}}$, b, and

S.E.$_b$ are plotted similarly against the computed values in figures 2B, C and D respectively. In each of these figures the line representing the regression of the estimated on the computed values, has been drawn. The equations for each of these regressions with the standard errors of the parameters are given in table 2.

TABLE 2

Regression of Estimated (Y) on Calculated (X) Values of the Parameters of Dosage-Effect Curves and Their Standard Errors

Value	$Y = \bar{y} + b(X - \bar{x})$	$\sigma_{y.x}$	σ_b	$\sigma_{\bar{y}}$
*Log E.D.$_{50}$	$Y = 0.539 + 1.013\,(X - 0.549)$	0.061	0.014	0.0085
†S.E.$_{\log E.D._{50}}$	$Y = 0.098 + 0.794\,(X - 0.098)$	0.010	0.025	0.0014
‡b	$Y = 3.518 + 0.786\,(X - 3.585)$	0.722	0.060	0.1001
§S.E.$_b$	$Y = 1.420 + 0.922\,(X - 1.222)$	0.465	0.116	0.0645

* Signifies median effective dose.
† Signifies standard error of median effective dose.
‡ Signifies slope constant.
§ Signifies standard error of slope constant.

DISCUSSION

It is evident from fig. 2A that the graphic estimates of the median effective dose are entirely satisfactory. On the other hand fig. 2C indicates that there was a definite tendency to overestimate b for low grade slopes and to underestimate its value for the steeper slopes. This tendency is verified by the appropriate regression equation in table 2, the regression coefficient of 0.786 differing significantly from the true value of 1.000.

Since the reciprocal of the slope constant appears in the formula for estimated S.E.$_{\log E.D._{50}}$ it may be expected that any error in the estimation of the slope constant will be transmitted. This may be partly offset, however, by the manner in which N' was selected and by ignoring the deviation of the average probit value from 5. Thus while we might have expected the regression coefficient for the estimated S.E.$_{\log E.D._{50}}$ on the computed values shown in table 2 and in fig. 2B to be greater than unity if the error in b were directly transmitted, it is actually 0.794. The formula for approximating the standard error of the slope appears to overestimate the value to some extent (table 2, and figure 2D) as was expected because of the manner in which N_s was determined.

It is apparent that the simple methods presented for obtaining the parameters of the dosage-effect curve and for approximating their standard errors give entirely satisfactory results at least with the type of biological data investigated here. Their usefulness can best be appreciated by comparing the time consumed in obtaining these approximate values with the time required to obtain them by mathematically fitting the curve. The former method requires about fifteen minutes, the latter one to two hours with the aid of a calculating machine. Cases may arise where greater accuracy is desired than is yielded by these approximations and one may then actually compute the parameters and their errors.

SUMMARY

The method of simple graphic solution of the dosage-effect curve, including approximate evaluation of the standard errors of the parameters has been presented and illustrated. These estimated values and their standard errors were compared with the corresponding values computed by Bliss's procedure for 54 dosage-effect curves. It was found that results obtained by graphic solution were in satisfactory agreement with those obtained by the more refined methods.

We wish to acknowledge the kindness of Dr. E. K. Marshall, Jr. in making freely available to us the data used in this study.

REFERENCES

(1) TREVAN: *Proceed. Royal Soc.* B, **101**: 483, 1927.
(2) GADDUM: *Medical Research Council, Special Report Series*, No. 183, 1933.
(3) DRAGSTEDT: *J. Pharmacol. & Exper. Therap.*, **32**: 215, 1928.
(4) BEHRENS: *Arch. exper. Path. u. Pharmak.*, **140**: 237, 1929.
(5) KÄRBER: *Arch. exper. Path. u. Pharmak.*, **162**: 480, 1931.
(6) BLISS: *Quart. J. Pharm. & Pharmacol.*, **11**: 192, 1938.
(7) LITCHFIELD, WHITE AND MARSHALL: *J. Pharmacol. & Exper. Therap.*, **67**: 437, 1939.
MARSHALL, LITCHFIELD AND WHITE: *J. Pharmacol. & Exper. Therap.*, **69**: 89, 1940.

A COMPARISON OF BALL-MILL AND COCA EXTRACTS OF RAGWEED POLLEN IN THE TREATMENT OF HAY-FEVER[1]

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Pollen extracts currently employed in this country in the treatment of pollen hay-fever and asthma are usually prepared according to the method described by Coca (1), in which the pollen cells are partially defatted with ether prior to extraction in a buffered saline medium. The ether-soluble lipoids, which are considered by many to be the principle cause of pollen dermatitis, are discarded. Since the pollen cells are intact at the end of this procedure (2), the materials obtained in the subsequent extraction are limited to those water soluble components which are freely diffusible through the cell wall. The possibility was considered that the actual disruption of the pollen cells prior to their extraction, and the elimination of the preliminary ether treatment, might assure a greater recovery of immunologically active substances from the interior of the pollen cell.

Comparative chemical analyses of Coca extracts of defatted but intact cells, and saline extracts of pollen previously ground in the Mudd-Czarnetzsky (3) low-temperature ball-mill lent initial support to this hypothesis. The ball-mill extracts gave consistently higher values for total solids, total nitrogen and especially protein nitrogen (2). Subsequent immunologic studies (4, 5) failed, however, to reveal any qualitative differences between the two types of extracts, for despite the differences in the method of preparation and in chemical composition, the antibody response in rabbits as measured by pre-

[1] This study was aided by a grant from Hynson, Westcott and Dunning, Inc., Baltimore, Md.

cipitins, complement fixing antibodies, and active and passive anaphylaxis were shown to be essentially equal. Similarly, there was no demonstrable difference in the reagin-neutralizing capacity (5) of ball-mill extracts and buffered saline extracts of defatted but intact pollen.

While these studies were in progress, it was considered desirable to determine the relative clinical value of the ball-mill and Coca extracts in the treatment of pollen hay-fever. This paper presents an analysis of the pertinent clinical data obtained in a group of ragweed hay-fever patients treated with the ball-mill extract prepared from short ragweed pollen, compared to a control group treated with buffered saline extracts of defatted whole pollen (Coca extract) prepared, however, from equal parts of giant and short ragweed pollen. The latter extract has been employed for more than a decade for the treatment of hay-fever in the Allergy Clinic at the Johns Hopkins Hospital. It is to be noted that the two extracts are not strictly comparable, since the ball-mill extract was prepared from short ragweed pollen only, while the Coca extract represented a mixture of short and giant ragweed pollen. However, this dissimilarity between the two extracts does not nullify the particular purpose of this study, namely, the attempt to determine whether short ragweed extract, prepared by the ball-mill method and containing a larger content of total and, especially, of protein nitrogen (Table I), would prove more valuable than the Coca extract in the treatment of pollen hay-fever.

PREPARATION OF AND CHEMICAL ANALYSES OF EXTRACTS

For the details of the preparation of the ball-mill extract, the reader is referred to our recent paper (2). Briefly, 5 grams of short ragweed pollen, pulverized at −80°C. in the ball-mill apparatus, was suspended in 100 cc. of physiologic salt solution. After extracting for one hour, the supernatant solution following centrifugation was sterilized by passage through a Berkfeld filter. In the case of the buffered defatted extract (5 per cent), prepared according to the method described by Coca (1), equal parts of giant and short ragweed extract were mixed prior to actual treatment of the patient. The values for total solids, total nitrogen and protein nitrogen of both extracts are given in Table I.

DETAILS OF TREATMENT

The preseasonal method of treatment was employed with each patient. Subcutaneous injections of extract were given biweekly, beginning with 0.1 cc. of a 1:10,000 dilution and increasing until the limit of the patient's tolerance was reached, or, until he was receiving concentrated extract. Injections of the maximum dosage attained were continued weekly, whenever possible, during the pollen season. The actual duration of treatment varied greatly. Some patients were treated continuously for 10 months, others for as short a period as one month. Similarly, the total amount of extract which each patient received, expressed either in cubic centimeters of concentrated extract or in protein nitrogen units, varied within wide limits (Table II).

All of the patients treated with short ragweed ball-mill extract were

TABLE I

	TOTAL SOLIDS	TOTAL NITROGEN	PROTEIN NITROGEN
	per cent	*per cent*	*per cent*
Ball-mill extract (prepared from short ragweed pollen)	1.74	0.092	0.058
Coca extract (equal parts of giant and short ragweed pollen)	1.00	0.079	0.025

patch-tested with the ether soluble lipoids obtained in the preparation of Coca's extract. No reactions were noted except in one case. This procedure was considered necessary to check the possible induction of skin sensitization to the lipoid which is considered to be the primary cause of pollen dermatitis, and of which there is a minute amount in the ball-mill extract of the whole pollen. None of the patients so treated complained of any symptoms suggesting cutaneous sensitization, and many retested at the conclusion of the course of treatment gave negative patch tests.

SELECTION OF PATIENTS

A total of 57 cases of ragweed hay-fever patients, all residing in or near Baltimore, were treated during 1938 with the short ragweed ball-mill extract. Of these, 17 had never previously received in-

TABLE II

Data on Cases of Hay-Fever Treated with Ball-Mill Extract Tabulated with Control Cases Treated with Coca Extract of Ragweed Pollen

(See Footnote and Text)

BALL-MILL EXTRACT						COCA'S EXTRACT					
Case	Age	Duration		Amt. of treatment protein nitrogen units	Result	Case	Age	Duration		Amt. of treatment protein nitrogen units	Result
		Symptoms	Treatment					Symptoms	Treatment		
		years						years			
1	53	20	6 yrs.	341,800	Excellent	1	39	20	6 yrs.	82,000	Good
2	60	22	22 yrs.	152,402	Excellent	2	60	31	2 yrs.	20,030	Good
3	39	18	4 yrs.	95,500	Excellent	3	37	33	2 yrs.	17,928	Excellent
4	14	8	1 yr.	79,460	Excellent	4	9	9	1 yr.	10,750	Excellent
5	41	13	2 yrs.	95,120	Excellent	5	40	10	5 yrs.	28,010	Fair
6	32	16	6 yrs.	48,669	Excellent	6	30	20	2 yrs.	25,500	Excellent
7	32	6	4 yrs.	37,120	Excellent	7	34	6	5 yrs.	45,000	Poor
8	57	9	5 yrs.	25,620	Excellent	8	51	9	5 yrs.	27,440	Good
9	55	5	2 yrs.	41,220	Excellent	9	33	6	2 yrs.	73,300	Excellent
10	58	29	4 yrs.	29,425	Excellent	10	47	25	2 yrs.	21,307	Excellent
11	35	6	4 yrs.	17,980	Excellent	11	34	5	4 yrs.	24,650	Excellent
12	39	4	2 yrs.	12,860	Excellent	12	45	5	1 yr.	8,191	Good
13	25	4	3 yrs.	19,952	Excellent	13	24	16	2 yrs.	51,870	Fair
14	44	9	2 yrs.	6,071	Excellent	14	36	16	1 yr.	5,631	Good
15	52	16	3 yrs.	219,994	Good	15	48	35	6 yrs.	66,510	Excellent
16	40	22	3 yrs.	85,500	Good	16	41	21	3 yrs.	42,300	Poor
17	17	11	6 yrs.	75,980	Good	17	16	11	2 yrs.	8,935	Good
18	35	25	2 yrs.	146,580	Good	18	24	2	4 yrs.	59,930	Good
19	27	8	7 yrs.	39,350	Good	19	22	12	4 yrs.	19,900	Good
20	50	23	6 yrs.	42,530	Good	20	29	12	8 yrs.	17,090	Excellent
21	9	4	½ yr.	24,617	Good	21	12	9	11 mo.	20,597	Good
22	59	29	2 yrs.	8,680	Good	22	50	18	7 yrs.	11,760	Excellent
23	12	4	½ yr.	9,802	Good	23	15	2	2 mo.	4,240	Fair
24	8	2	½ yr.	6,878	Good	24	9	3	1 yr.	1,000	Excellent
25	33	7	3 yrs.	2,523	Good	25	32	7	4 yrs.	21,720	Good
26	43	14	1 yr.	5,191	Good	26	54	6	5 yrs.	7,392	Good
27	28	6	4 yrs.	3,006	Good	27	36	6	4 yrs.	75,121	Good
28	54	40	7 yrs.	261,000	Fair	28	35	31	2 yrs.	37,750	Good
29	38	9	4 yrs.	102,240	Fair	29	38	16	1 yr.	5,631	Excellent
30	59	12	1 yr.	63,164	Fair	30	62	38	6 yrs.	8,940	Good
31	51	21	1 yr.	58,000	Fair	31	53	20	1 yr.	28,940	Poor
32	32	11	3 yrs.	68,440	Fair	32	32	8	2 yrs.	3,720	Fair
33	26	13	4 yrs.	1,727	Fair	33	62	15	8 yrs.	1,922	Fair
34	44	12	7 yrs.	58,100	Poor	34	44	7	2 yrs.	3,956	Good
35	48	7	4 yrs.	60,080	Poor	35	42	4	4 yrs.	30,300	Excellent
36	42	5	2 yrs.	45,680	Poor	36	42	7	2 yrs.	23,780	Fair

TABLE II—*Concluded*

BALL-MILL EXTRACT						COCA'S EXTRACT					
Case	Age	Duration		Amt. of treatment protein nitrogen units	Result	Case	Age	Duration		Amt. of treatment protein nitrogen units	Result
		Symptoms	Treatment					Symptoms	Treatment		
		years						*years*			
37	14	4	1 yr.	28,835	Poor	37	16	7	2 yrs.	19,012	Excellent
38	34	14	1 yr.	18,310	Poor	38	31	14	7 mo.	8,560	Excellent
39	36	30	12 yrs.	15,130	Poor	39	35	16	12 yrs.	22,170	Good
40	38	11	3 yrs.	10,440	Poor	40	38	15	3 yrs.	14,070	Excellent
41	34	10	9 mo.	265,408	Excellent	41	36	1	3 mo.	80,000	Good
42	32	1	13 mo.	70,780	Excellent	42	21	2	1 mo.	58,065	Good
43	49	3	9 mo.	141,073	Excellent	43	57	25	5 mo.	28,500	Excellent
44	42	12	0	68,440	Excellent	44	33	13	1 mo.	6,600	Good
45	43	8	8 mo.	43,795	Excellent	45	58	11	5 mo.	8,370	Good
46	30	6	6½ mo.	34,034	Excellent	46	44	5	2 mo.	30,500	Fair
47	61	40	12 mo.	7,163	Excellent	47	62	45	9 mo.	5,290	Good
48	28	3	3 mo.	3,662	Excellent	48	19	4	11 mo.	23,280	Excellent
49	32	1	13 mo.	237,199	Good	49	39	2	13 mo.	144,675	Good
50	20	3	10 mo.	66,700	Good	50	16	3	1 mo.	58,000	Good
51	21	7	6 mo.	32,886	Good	51	20	5	5 mo.	25,890	Good
52	14	7	12 mo.	16,128	Good	52	15	7	1 mo.	12,397	Excellent
53	34	5	6 mo.	13,982	Good	53	41	7	4 mo.	6,964	Good
54	49	1	10 mo.	13,271	Good	54	34	2	1 mo.	15,140	Excellent
55	15	2	1 mo.	3,625	Good	55	18	2	1 mo.	240	Fair
56	18	1	6 mo.	1,699	Good	56	14	1	3 mo.	15,950	Excellent
57	21	10	18 mo.	68,440	Fair	57	37	6	2 mo.	15,400	Excellent

With respect to age, duration of symptoms and duration of treatment, the two groups of patients have essentially similar distributions. Considering these variables independently, a no less divergent result would be obtained four times out of five on successive random samples from a single universe. With respect to the amount of treatment, however, the matching was not quite successful. Most of the patients treated with the larger amounts of extract happened to fall in the group treated with ball-mill extract; and although the average amount of treatment did not differ significantly in the two groups, the degree of divergence was greater than one would obtain in 9 out of 10 successive random samples from a single universe. However, this difference does not affect the conclusions of this paper. Despite the larger amount of treatment received, the patients on ball-mill extract did no better than the control group treated with Coca's extract.

jections of pollen extract; the remaining 40, however, had been treated during the previous year or years with Coca's extract. Since previous treatment may, and probably does, modify the response in many patients, these two groups have been differentiated in the following discussion and tables.

Since the total number of cases is relatively small, care was used in selecting control patients treated with Coca's extract. Of the many factors which may influence the symptoms, clinical course and therapeutic response of hay-fever, several may be controlled, namely, the age of the patient, the geographical location, the duration (number of seasons) of symptoms, the number of years of previous treatment, the total amount of extract administered (measured in protein nitrogen units) and the duration of treatment. In the present study, each case treated with ball-mill extract was eventually matched with a control case treated routinely in the Clinic with Coca's extract and so selected that the above mentioned factors approximated each other as closely as possible. The extent to which these matchings were successful is indicated in Table II and its footnote, in which is summarized a statistical analysis of the degree of correlation between these data.

RESULTS OF TREATMENT

The response of each patient to treatment, as judged by at least two observers in each case, was arbitrarily graded as excellent, good, fair and poor. Of the 57 patients treated with the ball-mill extract, 22 had an excellent response, 21 good, 7 fair and 7 poor. As shown in Table III, essentially the same response was obtained in the group treated with Coca's extract. In view of the small number of patients, the slight differences in results noted in the two groups cannot be considered statistically significant. One may conclude, therefore, that the ball-mill extract prepared from short ragweed pollen and containing appreciably greater amounts of total and, especially, of protein nitrogen was nevertheless no more valuable than the Coca extract in the treatment of ragweed hay-fever. Such a result is of particular significance in view of the facts 1) that most of the patients treated with larger amounts of extract were in the group receiving the ball-mill preparation (*cf.* footnote to Table II), and 2) that the ball-mill, unlike the Coca extract, was prepared from pure short ragweed pollen, considered by some (6) to be more active than the giant ragweed pollen. Despite this reputedly greater activity of the short ragweed and despite the higher protein content of ball-mill extract, the latter was no more active therapeutically than the Coca extract of combined giant and short ragweed pollen.

TABLE III

The Results of Treatment of Hay-Fever with the Ball-Mill Extract Compared to Coca Extract

	TOTAL NUMBER OF PATIENTS	CLINICAL RESULTS			
		Excellent	Good	Fair	Poor
Patients treated with ball-mill extract					
No previous treatment	17	8	8	1	0
Previous treatment with Coca's extract	40	15	13	6	7
Total	57	22	21	7	7
Patients treated with Coca extract					
No previous treatment	14	4	8	1	1
Previous treatment with Coca's extract	43	17	17	7	2
Total	57	21	25	8	3

Remarks:—Although the number of patients in each group is small, the trend of the results is essentially the same. The ball-mill extract proved to be as good as, but no better than, Coca's extract in the treatment of hay-fever.

TABLE IV

Data Illustrating Lack of Correlation between Total Amount of Pollen Extract, Duration of Treatment, Duration of Symptoms and Therapeutic Result

CLINICAL RESULTS	AMOUNT OF TREATMENT (PROTEIN NITROGEN UNITS)			DURATION OF TREATMENT (YEARS)			AGE			DURATION OF SYMPTOMS (YEARS)		
	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Ball-Mill Extract												
Excellent	4,662	341,800	36,746	¼	22	3.2	14	61	39.8	1	40	10.5
Good	1,699	219,994	22,167	1/12	7	1.8	9	59	28.6	1	29	8.2
Fair	1,727	261,000	40,022	1/12	7	3.7	21	59	41.1	9	40	13.5
Poor	10,400	68,440	18,200	1	12	4.3	14	48	36.5	4	30	11.8
Coca Extract (Control Group)												
Excellent	353	67,500	11,617	¼	7	1.8	9	50	29.6	1	33	10.4
Good	1,129	82,000	6,055	¼	12	2.2	11	60	36.5	6	40	12.1
Fair	1,920	59,900	13,036	¼	8	2.5	13	62	37.4	4	18	15.1
Poor	28,940	45,040	19,260	1/12	5	3.0	34	53	42.0	6	21	15.9

Although it is not germane to the present study, it is further of interest to point out (Table IV) that there was no regular correlation in this study between the total amount of pollen extract administered, the duration of treatment, the duration of symptoms and the therapeutic response.

SUMMARY

To the extent that one year's treatment and observation permit of general conclusions, the present study offers no evidence that ball-mill extracts of short ragweed pollen, prepared simply from disrupted cells, and containing appreciably higher concentrations of protein nitrogen are superior to buffered extracts of defatted but intact cells (Coca extract) in the treatment of ragweed hay-fever.

BIBLIOGRAPHY

1. Coca, A. F., Walzer, Matthew, and Thommen, A. A.: *Asthma and Hay-fever in Theory and Practice.* Charles C. Thomas, Springfield, 1931.
2. Eagle, Harry, Arbesman, Carl, and Winkenwerder, Walter L.: The Use of the Low Temperature Ball-Mill for the Preparation of Pollen Extracts. *Bull. Johns Hopkins Hospital*, **65**: 283–287, 1939.
3. Mudd, Stuart, Shaw, C. H., Czarnetzky, E. J., and Flosdorf, E. W.: A Low Temperature Ball-Mill for the Liberation of Labile Cellular Products. *Jour. Immunology*, **32**: 483, 1937.
4. Eagle, Harry, Arbesman, Carl, and Winkenwerder, Walter L.: The Production in Experimental Animals of Antibodies to Short Ragweed Pollen. *Jour. Immunology*, **36**: 425–435, 1939.
5. Winkenwerder, Walter L., Eagle, Harry, and Arbesman, Carl.: On the Presence in Rabbit Antisera vs. Ragweed Pollen of Skin-Sensitizing Antibodies Passively Transferable to Man. *Jour. Immunology*, **36**: 435–445, 1939.
6. Cooke, Robert A., Stull, Arthur, Selian, Herald and Barnard, James H.: Clinical and Serologic Study of the Relationship of Giant (Ambrosia Trifida) and Low (Ambrosia Artemisiaefolia) Ragweed Pollen. *Jour. Allergy*, **6**: 311, 1935.

BOOKS RECEIVED FOR REVIEW

Studies on Tuberculosis. American Journal of Hygiene Monographic Series, No. 16, February 1941. 198 pp. Illus. $1.10. *The Johns Hopkins Press, Baltimore, 1941.*

The Johns Hopkins Hospital. Dietary Department. Manual of Applied Nutrition. 86 pp. $1.00. *The Johns Hopkins Hospital Dietary Department, Baltimore, 1941.*

Applied Physiology. By SAMSON WRIGHT. 7th ed. 787 pp. Illus. $7.00. *Oxford University Press, New York, 1940.*

Pharmacology. By J. H. GADDUM. 407 pp. Illus. $6.00. *Oxford University Press, London: Humphrey Milford, 1940.* (*Oxford Medical Publications.*)

The Mask of Sanity. By HERVEY CLECKLEY. 298 pp. $3.00. *C. V. Mosby Company, St. Louis, 1941.*

Gastric and Duodenal Ulcers. By HAROLD AVERY. 110 pp. Illus. 7/6d. *John Bale & Staples, London, 1940.*

The Heart in Pregnancy and the Childbearing Age. By BURTON E. HAMILTON and K. JEFFERSON THOMSON. 402 pp. $5.00. *Little, Brown and Company, Boston, 1941.*

Neuro-Ophthalmology. By R. LINDSAY REA. 2nd ed. 688 pp. Illus. $13.50. *The C. V. Mosby Company, St. Louis, 1941.*

Physical Medicine. By FRANK H. KRUSEN. 846 pp. Illus. $10.00. *W. B. Saunders Company, Philadelphia and London, 1941.*

Cardiac Classics. A Collection of Classic Works on the Heart and Circulation with Comprehensive Biographic Accounts of the Authors. Fifty-Two Contributions by Fifty-One Authors. By FREDRICK A. WILLIUS and THOMAS E. KEYS. 858 pp. Illus. $10.00. *The C. V. Mosby Company, St. Louis, 1941.*

Introduction to Psychobiology and Psychiatry. A Textbook for Nurses. By ESTHER LORING RICHARDS. 357 pp. $2.50. *The C. V. Mosby Company, St. Louis, 1941.*

Fractures. By GEORGE PERKINS. 384 pp. Illus. $6.50. *Oxford University Press, London: Humphrey Milford, 1940.*

Practical Manual of Diseases of the Chest. By MAURICE DAVIDSON. 2nd ed. 575 pp. Illus. $13.50. *Oxford University Press, London: Humphrey Milford, 1941.*

Textbook of Pediatrics. By J. P. CROZER GRIFFITH and A. GRAEME MITCHELL. 3rd ed. 991 pp. Illus. $10.00. *W. B. Saunders Company, Philadelphia and London, 1941.*

Scabies—Civil and Military. By REUBEN FRIEDMAN. 288 pp. $3.00. *Froben Press, New York, 1941.*

Medical Diagnosis and Symptomatology. By SAMUEL A. LOEWENBERG. 5th ed. 1139 pp. Illus. $12.00. *F. A. Davis Company, Philadelphia, 1941.*

Oral Pathology. By KURT H. THOMA. 1306 pp. Illus. $15.00. *The C. V. Mosby Company, St. Louis, 1941.*

Manual of Allergy. By MILTON B. COHEN. 156 pp. $2.00. *Paul B. Hoeber, New York & London, 1941.*

Bacillary and Rickettsial Infections. By WILLIAM H. HOLMES. 676 pp. $6.00. *The Macmillan Company, New York, 1940.*

The Neuroses in War. Edited by EMANUEL MILLER. 250 pp. $2.50. *The Macmillan Company, New York, 1940.*

Synopsis of Materia Medica, Toxicology, and Pharmacology. By FORREST RAMON DAVISON. 633 pp. Illus. $5.00. *C. V. Mosby Company, St. Louis, 1940.*

Diseases Affecting the Vulva. By ELIZABETH HUNT. 215 pp. Illus. $4.00. *C. V. Mosby Company, St. Louis, 1940.*

Taber's Cyclopedic Medical Dictionary. By CLARENCE WILBUR TABER. 1488 pp. Illus. Thumb-indexed $3.00, Plain $2.50. *F. A. Davis Company, Philadelphia, 1940.*

Treatise on Medicolegal Ophthalmology. By ALBERT C. SNELL. 312 pp. Illus. $6.00. *C. V. Mosby Company, St. Louis, 1940.*

Text-Book of Ophthalmology. By SIR W. STEWART DUKE-ELDER. Vol. III, Diseases of the Inner Eye. 1373 pp. Illus. $18.50. *C. V. Mosby Company, St. Louis, 1941.*

Diagnosis and Treatment of Diseases of the Heart. By HENRY A. CHRISTIAN. (Reprinted from Oxford Monographs on Diagnosis and Treatment.) 599 pp. $7.00. *Oxford University Press, London, New York, Toronto, 1940.*

Bird Malaria. By REDGINAL HEWITT. (The American Journal of Hygiene Monographic Series, No. 15, July, 1940.) 228 pp. Illus. $1.10. *The Johns Hopkins Press, Baltimore, 1940.*

Psychotherapy. By LEWELLYS F. BARKER. 218 pp. $2.00. *D. Appleton-Century Company, New York & London, 1940.*

L. Emmett Holt, Pioneer of a Children's Century. By R. L. DUFFUS and L. EMMETT HOLT, JR. 295 pp. Illus. $3.00. *D. Appleton-Century Company, New York & London, 1940.*

Methods of Treatment. By LOGAN CLENDENING and EDWARD H. HASHINGER. 7th ed. 997 pp. Illus. $10.00. *C. V. Mosby Company, St. Louis, 1941.*

Foreign Bodies Left in the Abdomen. By HARRY STURGEON CROSSEN and DAVID FREDERIC CROSSEN. 762 pp. Illus. $10.00. *C. V. Mosby Company, St. Louis, 1940.*

Pathological Histology. By ROBERTSON F. OGILVIE. 332 pp. Illus. $8.50. *Williams & Wilkins Company, Baltimore, 1940.*

Lipidoses: Diseases of the Cellular Lipid Metabolism. By SIEGFRIED J. THANNHAUSER. Edited by HENRY A. CHRISTIAN. (Reprinted from Oxford Loose-Leaf Medicine.) 370 pp. Illus. $6.00. *Oxford University Press, New York [etc.], 1940.*

The Endocrine Function of Iodine. By WILLIAM THOMAS SALTER. 351 pp. Illus. $3.50. *Harvard University Press, Cambridge, Mass., 1940.*